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 NEWS  3   JAN 27  Source of Registration (SR) information in REGISTRY updated
                   and searchable
 NEWS  4   JAN 27  A new search aid, the Company Name Thesaurus, available in
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 NEWS  5   FEB 05  German (DE) application and patent publication number format
                   changes
 NEWS  6   MAR 03  MEDLINE and LMEDLINE reloaded
 NEWS  7   MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS  8   MAR 03  FRANCEPAT now available on STN
 NEWS  9   MAR 29  Pharmaceutical Substances (PS) now available on STN
 NEWS 10   MAR 29  WPIFV now available on STN
 NEWS 11   MAR 29  New monthly current-awareness alert (SDI) frequency in RAPRA
 NEWS 12   APR 26  PROMT: New display field available
 NEWS 13   APR 26  IFIPAT/IFIUDB/IFICDB: New super search and display field
                   available
 NEWS 14   APR 26  LITALERT now available on STN
 NEWS 15   APR 27  NLDB: New search and display fields available
 NEWS 16   May 10  PROUSDDR now available on STN
 NEWS 17   May 19  PROUSDDR: One FREE connect hour, per account, in both May
                   and June 2004
 NEWS 18   May 12  EXTEND option available in structure searching
 NEWS 19   May 12  Polymer links for the POLYLINK command completed in REGISTRY
 NEWS 20   May 17  FRFULL now available on STN
 NEWS 21   May 27  STN User Update to be held June 7 and June 8 at the SLA 2004
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 NEWS 22   May 27  New UPM (Update Code Maximum) field for more efficient patent
                   SDIs in CAplus
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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
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FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004

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FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 17 Jun 2004 (20040617/PD)
FILE LAST UPDATED: 17 Jun 2004 (20040617/ED)
HIGHEST GRANTED PATENT NUMBER: US6751803
HIGHEST APPLICATION PUBLICATION NUMBER: US2004117887
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ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 17 Jun 2004 (20040617/PD)
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              1 "HALLOWITZ ROBERT"/IN
              6 "HALLOWITZ ROBERT A"/IN
L1            7 "HALLOWITZ ROBERT"/IN OR "HALLOWITZ ROBERT A"/IN

=> d l1,ti,1-7

L1   ANSWER 1 OF 7  USPATFULL on STN
TI     Methods for characterizing the viral infectivity status of a host

L1   ANSWER 2 OF 7  USPATFULL on STN
TI     METHODS OF IMPROVING INFECTIVITY OF CELLS FOR VIRUSES

L1   ANSWER 3 OF 7  USPATFULL on STN
TI     METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD

L1   ANSWER 4 OF 7  USPATFULL on STN
TI     REAGENT SYSTEM AND KIT FOR DETECTING HIV INFECTED CELLS
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L1   ANSWER 5 OF 7  USPATFULL on STN
TI     Method of and apparatus for automating detection of microorganisms

L1   ANSWER 6 OF 7  USPATFULL on STN
TI     Reagent system for detecting HIV-infected peripheral blood lymphocytes
       in whole blood

L1   ANSWER 7 OF 7  USPATFULL on STN
TI     Cartridge test system for the collection and testing of blood in a
       single step


=> d l1,cbib,ab,clm,1-4,6

L1   ANSWER 1 OF 7  USPATFULL on STN
2002:185564 Methods for characterizing the viral infectivity status of a host.
    Hallowitz, Robert A., Newmarket, MD, UNITED STATES
    Krowka, John, Frederick, MD, UNITED STATES
    Matlock, Shawn, Frederick, MD, UNITED STATES
    Bio-Tech Imaging, Inc., Frederick, MOLDOVA, REPUBLIC OF (U.S. corporation)
    US 2002098476 A1 20020725
    APPLICATION: US 2001-893604  A1  20010629 (9)
    PRIORITY: US 2000-215075P  20000630 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Methods in accordance with the present invention involve novel
       measurements of the disease status of hosts infected with the human
       immunodeficiency virus. In particular, the present invention relates to
       a measurements of the numbers in a sample volume of (a) productively
       HIV-infected cells and (b) cells capable of being infected by HIV, e.g.,
       cells expressing CD4, CCR5, and/or CXCR4. These two values can be
       represented as a single ratio, e.g., number of productively infected
       cells/number of cells capable of being infected by HIV, and can be
       utilized as an indicator of disease status, such as disease progression,
       viral replication, etc.
CLM    What is claimed is:
       1. A method of assessing the infectivity status of a host infected with
       HIV, comprising:  measuring the number of cells in a sample which are
       expressing cell-surface gp120 and the number of lymphocytes in said
       sample which are CD4 positive, whereby the infectivity status of the
       host is assessed.

       2. A method of claim 1, wherein the infectivity status is represented by
       the number of cells expressing cell-surface gp120 per unit volume
       divided by the number of cells which are CD4 positive per unit volume.

       3. A method of claim 1, wherein the measuring is accomplished by flow
       cytometry.

       4. A method of claim 1, wherein the measuring is accomplished by a
       fluorescence resonance energy transfer assay.

       5. A method of claim 1, wherein the cells are peripheral blood
       mononuclear cells.

       6. A method of claim 1, further comprising:  combining an effective
       amount of an anti-gp120 antibody attached to a first detectable label
       and an effective amount of an anti-CD4 antibody attached to a second
       detectable label under conditions effective for said antibodies to bind
       gp120 and CD4 respectively.

       7. A method of claim 6, wherein said measuring is accomplished by flow
       cytometry.

       8. A method of claim 1, further comprising:  combining an effective
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amount of an anti-gp120 antibody attached to a detectable label, an effective amount of an antibody specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said gp120 to form a mixture, wherein said antibody specific-for said detectable label is attached to a magnetic particle; incubating said mixture under conditions effective for binding of said anti-gp120 antibody to gp120 on said cells, and, for binding of said antibody specific-for said detectable label to said detectable label attached to said anti-gp120 antibody, to form a complex, wherein said anti-gp120 antibody is bound to said gp120 displayed on a viral-infected cell; separating said complex by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field, and determining the presence of magnetically-separated cells by detecting said detectable label, whereby said magnetically separated cells are lymphocytes expressing cell-surface gp120.

9. A method of claim 1, wherein the CD4 count of said host is less than $200/mm^3$ of whole blood.

10. A method of claim 1, wherein the host has been treated with HAART.

11. A method of determining the infectivity status of a host infected with HIV virus who has tested negative in a virus co-culture assay, comprising: measuring the fraction of lymphocytes expressing cell-surface gp120 and the fraction of lymphocytes which are CD4 positive, whereby the infectivity status of the host is assessed.

12. A method of claim 11, wherein the measuring is accomplished by flow cytometry.

13. A method of claim 11, wherein the measuring is accomplished by a fluorescence resonance energy transfer assay.

14. A method of claim 11, wherein the cells are peripheral blood mononuclear cells.

15. A method of claim 11, further comprising: combining an effective amount of an anti-gp120 antibody attached to a first detectable label and an effective amount of an anti-CD4 antibody attached to a second detectable label under conditions effective for said antibodies to bind gp120 and CD4 respectively.

16. A method of claim 15, wherein said measuring is accomplished by flow cytometry.

L1 ANSWER 2 OF 7 USPATFULL on STN
2002:66846 METHODS OF IMPROVING INFECTIVITY OF CELLS FOR VIRUSES.
HALLOWITZ, ROBERT A., GAITHERSBURG, MD, UNITED STATES
YOUNG, SUSAN, ALBUQUERQUE, NM, UNITED STATES
KING, CHESTER, FREDERICK, MD, UNITED STATES
US 2002037498 A1 20020328
APPLICATION: US 1999-299625 A1 19990427 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to cells which have improved receptivity to viruses which are capable of infecting them. Receptivity to such viruses is improved by selecting cells from a population which express the receptor(s) that enable a virus to attach to the cell and gain entry into it. Any combination of viruses and host cell lines can be used.

In a preferred embodiment, the present invention relates to improving receptivity or infectivity of a cell line which can be infected with an immunodeficiency virus, such as HIV-1. Especially preferred embodiments of the invention relate to methods of improving (or assaying for) the infectivity for HIV-1 in a HIV-1 receptive cell line, preferably a

continuous cell line transformed with DNAs coding for expressible CD4 and expressible HIV-1 coreceptor, comprising, in any effective order, a) isolating the cells expressing CD4 and an HIV-1 coreceptor on their cell surface; b) contacting the isolated cells with HIV-1 under conditions effective for the HIV-1 to infect the cells; and c) detecting the number of cells infected with HIV-1, thereby assaying for infectivity of HIV-1. This method facilitates the measurement of true infectivity and infectivity reduction values by quantifying the percentage of infected cells in the population of specific cells capable of being infected by virus, rather than in a population of mixed cells, only some which are capable of being infected.

CLM What is claimed is:

1. A method of obtaining HIV-receptive cells, comprising: a) labeling cells with a CD4 binding reagent and a HIV-1 co-receptor binding reagent; and b) isolating cells which are labeled with said CD4 binding reagent and said HIV-1 co-receptor binding reagent, wherein said cells are receptive to HIV-1 infection.

2. A method of obtaining HIV-receptive cells, comprising: a) isolating cells labeled with a CD4 binding reagent and a HIV-1 coreceptor binding reagent.

3. A method of assaying for the infectivity of HIV-1 in a continuous cell line transformed with DNAs coding for expressible CD4 and expressible HIV-1 coreceptor, comprising: a) isolating said cells expressing said DNAs coding for expressible CD4 and expressible HIV-1 coreceptor; b) contacting said isolated cells with HIV-1 under conditions effective for said HIV-1 to infect said cells; and c) detecting the number of cells infected with HIV-1, thereby assaying for infectivity of HIV-1.

4. A method of claim 3, wherein said continuous cell line is a human cell line.

5. A method of claim 4, wherein said human cell line is a HeLa cell line.

6. A method of claim 5, wherein said HeLa cell line is MAGI-CCR5.

7. A method of claim 3, wherein isolating comprises: a) labeling said cells transformed with DNAs coding for expressible CD4 and expressible HIV-1 coreceptor with a CD4 binding reagent; b) separating out CD4 binding reagent labeled cells; c) labeling said cells transformed with DNAs coding for expressible CD4 and expressible HIV-1 coreceptor with a HIV coreceptor binding reagent; and d) separating out HIV-1 coreceptor binding reagent labeled cells.

8. A method of claim 7, wherein separating is by positive selection using immunomagnetic or fluorescence-activated cell sorting.

9. A method of claim 7, wherein said binding reagents are antibodies comprising a capture-moiety.

10. A method of claim 7, wherein said CD4 binding reagent is a FITC-conjugated CD4-specific antibody.

11. A method of claim 7, wherein said HIV-1 coreceptor binding reagent is a FITC-conjugated HIV-1-coreceptor specific antibody.

12. A method of claim 7, wherein said HIV-1 coreceptor is CCR5.

13. A method of claim 7, wherein: said (a) labeling comprises: contacting said cells with a CD4 binding reagent which is FITC-conjugated CD4-specific antibody under conditions effective for antibody to label cell-surface CD4; and said (b) separating out comprises: contacting cell-surface labeled cells with anti-FITC

antibody magnetic particles under conditions effective for said antibody to attach to said CD4-specific antibody on said cell-surface; applying a magnetic field to said labeled cells which is effective to retain said magnetic particles; and eluting the retained particles to form a sample of separated out cells.

14. A method of claim 7, wherein: said (c) labeling comprises: contacting said cells with a HIV-1 coreceptor binding reagent which is FITC-conjugated HIV-1 coreceptor-specific antibody under conditions effective for antibody to label cell-surface HIV-1 coreceptor; and said (d) separating out comprises: contacting cell-surface labeled cells with anti-FITC antibody magnetic particles under conditions effective for said antibody to attach to said HIV-1 coreceptor-specific antibody on said cell-surface; applying a magnetic field to said labeled cells which is effective to retain said magnetic particles; and eluting the retained particles to form a sample of separated out cells.

15. A method of claim 14, wherein said HIV-1 coreceptor is CCR5.

16. A method of claim 7, wherein said continuous cell line is a human cell line.

17. A method of claim 7, wherein said human cell line is a HeLa cell line.

18. A method of claim 7, wherein said HeLa cell line is MAGI-CCR5.

L1 ANSWER 3 OF 7 USPATFULL on STN

2001:199904 METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD.

HALLOWITZ, ROBERT, GAITHERSBURG, MD, United States
SALAS, VIRGINIA, ALBUQUERQUE, NM, United States

US 2001039007 A1 20011108
APPLICATION: US 1999-296534 A1 19990422 (9)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a new HIV status of a patient called "latent viral load." To measure the "latent viral load," in accordance with a preferred embodiment of the present invention, a population of sample cells is obtained from a desired source, such as an infected patient. The sample cell population is depleted of overtly infected cells and cells harboring active virus, to produce a subset of "resting cells" comprising uninfected and latently-infected cells. This subset is treated with an agent and/or condition that activates the latent virus in the host cell genome and results in a productive infection. The thus-produced infection reflects the "latent viral load" of the host because it reveals the presence of quiescent virus in cells. The latent viral load is useful in assessing a patient's disease status and the efficacy of highly active antiretroviral therapy and other treatment protocols.

CLM What is claimed is:

1. A method of determining the latent viral load in a host infected with HIV comprising, treating resting lymphoid mononuclear cells obtained from the host with an effective amount of an agent capable of activating an HIV virus integrated into the genome of the cells; and detecting the expression of cell-surface gp120 after the cells have been treated with the agent, wherein the presence or amount of cells expressing cell-surface gp120 is a measure of latent viral load.

2. A method of claims 1, further comprising obtaining the resting lymphoid mononuclear cells by the steps of: a) obtaining a sample cell population; b) depleting the sample cell population of cells expressing cell-surface gp120; and c) depleting sample cell population of cells expressing HLA-DR.

3. A method of claim 2, wherein the sample cells are depleted of gp120

expressing cells by the steps of: a) contacting sample cells with gp120-specific antibodies, each conjugated to a capture moiety, under conditions effective for the antibodies to attach to gp120 on the surface of cells, thereby forming labeled-cells; b) contacting the labeled-cells with capture moiety-specific antibody under conditions effective for the capture moiety-specific antibody to attach to the labeled-cells, thereby forming a complex-labeled cells; and c) removing the complex-labeled cells, thereby depleting sample cells of gp120+ cells.

4. A method of claim 3, wherein the capture moiety-specific antibody is conjugated to magnetic particles.

5. A method of claim 3, wherein the capture moiety is FITC and the capture moiety-specific antibody is FITC-specific antibody conjugated to a magnetic bead.

6. A method of claims 4, wherein the magnetic particles are 10-100 nm in diameter.

7. A method of claims 5, wherein the magnetic particles are 10-100 nm in diameter.

8. A method of claims 3, wherein the removing is accomplished by a magnetic field acting on the magnetic particles.

9. A method of claim 2, further comprising: separating CD4+ cells from the sample.

10. A method of claim 2, further comprising: separating CD8+ cells from the sample.

11. A method of claim 2, wherein the depleting sample cell population of cells expressing HLA-DR is accomplished by flow cytometry cell sorting and said cells are labeled with a fluorochrome-labeled antibody specific-for HLA-DR.

12. A method of claim 1, wherein the tissue is lymphoid.

13. A method of claims 1, wherein the agent is phorbol ester or a cytokine.

14. A method of claim 1, wherein the measure of latent viral load is number of cells expressing gp120 after treating the resting with an effective amount of an agent capable of activating an HIV virus integrated into the genome of the cells.

15. A method of claim 1, wherein the measure of latent viral load is compared to an established cell line harboring latent HIV-1.

16. A method of claim 15, wherein the cell line is OM-10.1, U1, or Jurkat cells.

17. A method of treating a viral infection comprising measuring the latent viral load in an HIV-infected patient; and determining whether to administer to the patient an agent capable of activating an HIV virus integrated into the genome of a cell by the value of the latent viral load.

L1 ANSWER 4 OF 7 USPATFULL on STN
2001:114495 REAGENT SYSTEM AND KIT FOR DETECTING HIV INFECTED CELLS.

KING, CHESTER F., FREDERICK, MD, United States
HALLOWITZ, ROBERT A., GAITHERSBURG, MD, United States

US 2001008760 A1 20010719
APPLICATION: US 1998-139663 A1 19980825 (9)

WO 1997-US18649 19971015 None PCT 102(e) date
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to blood collection and diagnostics. More particularly, the invention relates to blood collection and diagnostics utilizing techniques such as magnetic separation and photodetection. The present invention also relates to methods and an apparatus for detecting the presence of antigens displayed on the surface of cells. More preferably, the present invention relates to the detection of cells infected by human immunodeficiency virus (HIV) and related viruses. In accordance with the present invention, HIV-infected cells can be detected and separated from uninfected cells. In a preferred embodiment, separation is achieved by a magnetic field. By coating the infected cells with magnetic particles, transfer of the cells to a precise location is facilitated. A novel aspect of the present invention is a cartridge antigen test which allows for the collection and mixing of blood with reagents in one package, which can be viewed on a fluorescent microscope.

CLM What is claimed is:

1. A method of separating cells expressing a viral antigen, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the mixture, a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to the viral antigen on the cell surface; c) adding to the mixture resulting from b), a second binding partner specific for the first binding partner and attached to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and d) separating target cells containing the complex, whereby said separation is achieved by a magnetic field.

2. A method of claim 1, further comprising adding to the target cell a sample antibody specific for the viral antigen.

3. A method of claim 2, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample antibody

4. A method of claim 1, further comprising adding to the target cell a sample comprising an antibody specific for the viral antigen, whereby the amount of the second antibody is effective for interfering with the binding of the first binding partner to the viral antigen.

5. A method of claim 1, further comprising adding to the target cell a sample suspected of containing an antibody specific for the viral antigen.

6. A method of claim 5, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample.

7. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen.

8. A method of claim 6, wherein the second binding partner is an antibody specific for the first binding partner.

9. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen, which antibody is labeled with a detectable label.

10. A method of claim 9, wherein the second binding partner is an antibody specific for the detectable label.

11. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen, which antibody is labeled with a detectable label.

12. A method of claim 6, wherein the virus is HIV.

13. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen gp120, which antibody is labeled with a detectable label; and the second binding partner is an antibody specific for the detectable label.

14. A method of claim 6, wherein the target cell is a T-cell line.

15. A method of claim 6, wherein the sample is a body fluid or blood.

16. A method of claim 6, wherein measurement of the number of target cells separated in d) in the presence and absence of the sample is accomplished by flow cytometry.

17. A method of claim 12, wherein the first binding partner is a receptor for the viral antigen.

18. A method of claim 16, wherein the first binding partner is a receptor for the viral antigen and is labeled with a detectable label; and the second binding partner is an antibody specific for the detectable label.

19. A method of claim 6, wherein the bead diameter is about 50-120 nm.

20. A method of claim 6, wherein the cell is contacted by at least about 100-1000 beads.

21. A method of identifying an agent which interferes with viral infection of a cell, a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test sample containing an agent suspected with interfering with viral infection of the test cell; c) adding to the mixture of b), a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen on the cell surface; d) adding to the resultant mixture formed in c), a second binding partner specific for the first binding partner and to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; e) separating test cells containing said complex, whereby said separation is achieved by a magnetic field; and f) determining the number of cells infected with said virus in the presence and the absence of said test agent.

22. A magnetic bead having a surface coated by a cell-surface virus receptor for HIV.

23. A magnetic bead of claim 21, wherein the virus receptor is CD4.

24. A method of separating virus-infected cells from non-virus infected cells in a sample comprising, combining (a) a first antibody recognizing a viral antigen on the surface of said cell and attached to a magnetic particle; (b) a second antibody recognizing said viral antigen on the surface of said cell and attached to a detectable label; and (c) a sample containing said virus-infected cells, to form a mixture; incubating said mixture under conditions effective for binding of said antibodies to said viral antigen to form a complex, said complex comprising said first and second antibody bound to said virus-infected cell, and moving said magnetic particle to a predetermined point on a

reaction vessel holding said mixture, whereby said moving is accomplished by a magnetic field acting on said magnetic particle resulting in separating said virus-infected cells from non-virus infected cells, wherein said moving is accomplished without removing unbound antibody first and second antibody from said mixture.

25. A method of claim 24, further comprising detecting the label of said second antibody bound to said viral antigen on said virus-infected cell, wherein said first and second antibody recognize different epitopes of said viral antigen.

26. A method of separating cells infected with a virus, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus; b) fixing and permeabilizing said cells; c) adding to the fixed and permeabilized cells, a first binding partner specific for an antigen coded for by the virus, which viral antigen is ultimately expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to said viral antigen on the inside of said fixed and permeabilized cell; d) adding to the result of c), a second binding partner specific for the first binding partner and attached to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and e) separating target cells containing the complex, whereby said separation is achieved by a magnetic field.

27. A method of identifying an agent which interferes with viral infection of a cell, comprising: a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test agent suspected with interfering with viral infection of the test cell; c) fixing and permeabilizing said cells; d) adding a first binding partner specific for an antigen coded for by the virus, which viral antigen is expressed ultimately on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen when said viral antigen is expressed in the interior of said cell; e) adding to the resultant mixture formed in d), a second binding partner specific for the first binding partner and to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; f) separating test cells containing said complex, whereby said separation is achieved by a magnetic field; and g) determining whether the test sample changes the number of test cells containing the complex when compared to the process performed in the absence of said agent.

28. A method claim 27, where said test agent is added to cells prior to simultaneous to contacting cell with said test agent.

29. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen antibody attached to a detectable label, an effective amount of an antibody specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture, wherein said antibody specific-for said detectable label is attached to a magnetic particle; b) incubating said mixture under conditions effective for binding of said anti-cell surface viral antibody to said cell-surface viral antigen, and, for binding of said antibody specific-for said detectable label to said detectable label attached to said anti-cell surface viral antibody, to form a complex, wherein said anti-viral antibody is bound to said cell-surface antigen displayed on a viral-infected cell; and c) separating said complex, comprising said cells expressing said cell-surface viral antigen and magnetic particles, by applying a

magnetic field to said mixture, whereby said complex is retained by said magnetic field.

30. A method of claim 29, wherein viral-infected cells are infected with HIV.

31. A method of claim 29, wherein said cell-surface viral antigen is an envelope glycoprotein for HIV.

32. A method of claim 29, wherein the envelope glycoprotein is gp120 or gp41.

33. A method of claim 29, wherein said anti-cell surface viral antibody is a polyclonal antibody specific for HIV envelope glycoprotein and said viral-infected cells are infected with HIV.

34. A method of claim 29, wherein said detectable label is FITC, TRITC, or R-phycoerthryin.

35. A method of claim 29, further comprising counting said magnetically-separated cells by flow cytometry.

36. A method of claim 29, wherein said magnetic particles are about 10-150 nm in diameter. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen antibody attached to a magnetic particle and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture; b) incubating said mixture under conditions effective for binding of said anti-cell surface viral antibody to said cell-surface viral antigen displayed on said viral-infected cells, to form a complex; and c) separating said complex comprising said cells expressing said cell-surface viral antigen and magnetic particles by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field.

L1 ANSWER 6 OF 7 USPATFULL on STN

1998:122214 Reagent system for detecting HIV-infected peripheral blood lymphocytes in whole blood.

King, Chester F., Frederick, MD, United States
Hallowitz, Robert A., Gaithersburg, MD, United States
The Avriel Group, AMCAS Division Inc., United States (part interest) a part interest

US 5817458 19981006
APPLICATION: US 1996-732782 19961015 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Fluorometric immunological assay method for detection of HIV-1 infection in which Murine anti-gp120 monoclonal antibodies coupled to paramagnetic microspheres (14) and Fluorescein conjugated anti-gp120 polyclonal antibodies IgG (16) are incubated in a few drops of whole blood diluted in 0.5 cc phosphate buffered saline (10). After incubation for 5 minutes, the HIV-infected peripheral blood lymphocytes (18) will be coated with both the Murine anti-gp120 monoclonal antibodies coupled to paramagnetic microspheres (14) and Fluorescein conjugated anti-gp120 polyclonal antibodies IgG (16) at exposed gp120 antigens (20) binding sites. At the time of measurement said HIV- infected peripheral blood lymphocytes (18) will be pulled against the wall of the measurement vessel by means of a magnetic gradient (26). The cells adhering to the vessel wall are illuminated at 488 nm monochromatic light by a focused light source (28) and the resultant emitted fluorescence is imaged, measured and recorded.

CLM What is claimed is:

1. A method of detecting an HIV-infected cell in an aqueous sample comprising the steps of, a) combining a first anti-gp120 antibody

attached to a magnetic particle; a second anti-gp120 antibody attached to a detectable label; and an aqueous sample containing HIV-infected peripheral blood lymphocytes displaying gp120 on the cell surface, to form a mixture; b) incubating said mixture under conditions effective for binding of said antibodies to said gp120 to form a complex, said complex comprising said first and second antibody bound to a HIV-infected cell on said magnetic particle; and c) moving said magnetic particle to a predetermined point on a reaction vessel holding said mixture, wherein said moving is accomplished by a magnetic field acting on said magnetic particle; d) detecting the label of said second antibody bound to gp120 on said HIV-infected cell, with the proviso that no step of washing of said mixture and no step of removing unbound first antibody and unbound second antibody from said mixture is performed in steps a), b), c), and d).

2. A method of claim 1, wherein said first and second antibody recognize different regions of gp120.

3. A method of claim 1, wherein said aqueous sample is whole blood.

4. A method of claim 1, wherein said predetermined point is illuminated with a light effective to detect said label.

5. A method of claim 1, wherein said detectable label is FITC.

6. A method of claim 1, wherein said first antibody is a monoclonal antibody.

7. A method of claim 1, wherein said second antibody is a polyclonal antibody.

8. A method of claim 1, where said first antibody is a monoclonal antibody and said second antibody is a polyclonal antibody coupled to a detectable label which is FITC.

9. A method of detecting an HIV-infected cell in an aqueous sample comprising the steps of, a) combining a first anti-gp120 antibody attached to a magnetic particle; a second anti-gp120 antibody attached to a detectable label; and an aqueous sample containing HIV-infected cells displaying gp120 on the cell surface, to form a mixture; b) incubating said mixture under conditions effective for binding of said antibodies to said gp120 to form a complex, said complex comprising said first and second antibody bound to a HIV-infected cell on said magnetic particle; and c) moving said magnetic particle to a predetermined point on a reaction vessel holding said mixture, wherein said moving is accomplished by a magnetic field acting on said magnetic particle; d) detecting the label of said second antibody bound to gp120 on said HIV-infected cell, with the proviso that no step of washing of said mixture and no step of removing unbound first antibody and second antibody from said mixture is performed in a), b), c), and d).

10. A method of claim 1, wherein said HIV-infected cell is a peripheral blood lymphocyte.

11. A method of claim 9, wherein said first and second antibody recognize different regions of gp120.

12. A method of claim 9, wherein said aqueous sample is whole blood.

13. A method of claim 9, wherein said predetermined point is illuminated with a light effective to detect said label.

14. A method of claim 9, wherein said detectable label is FITC.

15. A method of claim 9, wherein said first antibody is a monoclonal antibody.

16. A method of claim 9, wherein said second antibody is a polygonal antibody.

17. A method of claim 9, where said first antibody is a monoclonal antibody and said second antibody is a polyclonal antibody coupled to a detectable label which is FITC.

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=>
=> d his

     (FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
                E HALLOWITZ R A/IN
L1            7 S E4 OR E5

=> e krowka john/in
E1            2      KROWICKI KRZYSZTOF/IN
E2            2      KROWIORZ JOSEF/IN
E3            1 --> KROWKA JOHN/IN
E4            1      KROWL GUY/IN
E5            6      KROWL THOMAS R/IN
E6            1      KROWL THOMOS R/IN
E7            1      KROWL WILLIAM G/IN
E8            3      KROWNE CLIFFORD M/IN
E9            1      KROWORSCH HANS PETER/IN
E10           2      KROY RALPH E/IN
E11          17      KROY WALTER/IN
E12           5      KROYAN ARMEN/IN

=> s e3
L2            1 "KROWKA JOHN"/IN

=> d l2,ti

L2   ANSWER 1 OF 1  USPATFULL on STN
TI     Methods for characterizing the viral infectivity status of a host


=> e matlock shawn/in
E1            1      MATLOCK ROY L/IN
E2            1      MATLOCK SELDEN W/IN
E3            1 --> MATLOCK SHAWN/IN
E4            1      MATLOCK SHAWN A/IN
E5            1      MATLOCK TEENIE GAIL/IN
E6            1      MATLOCK THOMAS D/IN
E7            1      MATLOCK TONY L/IN
E8            2      MATLOCK WALLACE M/IN
E9            1      MATLOCK WAYNE/IN
E10           4      MATLOCK WILLIAM C/IN
E11           1      MATLOFF GREGORY L/IN
E12           1      MATLOFF NORMAN/IN

=> s e3 or e4
              1 "MATLOCK SHAWN"/IN
              1 "MATLOCK SHAWN A"/IN
L3            2 "MATLOCK SHAWN"/IN OR "MATLOCK SHAWN A"/IN

=> d l3,ti,1-2

L3   ANSWER 1 OF 2  USPATFULL on STN
TI     Methods for characterizing the viral infectivity status of a host
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L3   ANSWER 2 OF 2  USPATFULL on STN
TI     METHOD OF PREPARING CRYOGENICALLY PRESERVED ADHERENT CELL CONTAINING
       PLATE FOR TISSUE CULTURE APPLICATIONS


=> s (HIV or human immunodeficiency virus)
         31369 HIV
        391063 HUMAN
         18098 IMMUNODEFICIENCY
         74523 VIRUS
         12962 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L4       33048 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l4 and (gp120 or gp160)
          3442 GP120
          1203 GP160
L5        3422 L4 AND (GP120 OR GP160)

=> s l5 and (CD4?)
         20492 CD4?
L6        2490 L5 AND (CD4?)

=> s l6 and (FRET or RET or fluorescent resonance energy transfer or resonance energy transfer)
          3229 FRET
          4121 RET
        119830 FLUORESCENT
        116374 RESONANCE
        687797 ENERGY
        756035 TRANSFER
           267 FLUORESCENT RESONANCE ENERGY TRANSFER
                 (FLUORESCENT(W)RESONANCE(W)ENERGY(W)TRANSFER)
        116374 RESONANCE
        687797 ENERGY
        756035 TRANSFER
          2343 RESONANCE ENERGY TRANSFER
                 (RESONANCE(W)ENERGY(W)TRANSFER)
L7         182 L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER OR
               RESONANCE ENERGY TRANSFER)

=> s l7 and antibod?
        103369 ANTIBOD?
L8         182 L7 AND ANTIBOD?

=> s l8 and (gp120/clm or gp160/clm)
           389 GP120/CLM
           150 GP160/CLM
L9          16 L8 AND (GP120/CLM OR GP160/CLM)

=> d l9,ti,1-16

L9   ANSWER 1 OF 16  USPATFULL on STN
TI     Uses of a chemokine receptor for inhibiting HIV-1 infection

L9   ANSWER 2 OF 16  USPATFULL on STN
TI     Compositions and methods for inhibition of HIV-1 infection

L9   ANSWER 3 OF 16  USPATFULL on STN
TI     Potent oncolytic herpes simplex virus for cancer therapy

L9   ANSWER 4 OF 16  USPATFULL on STN
TI     HIV-1 group O antigens and uses thereof

L9   ANSWER 5 OF 16  USPATFULL on STN
TI     Compositions and methods for inhibition of hiv-1 infection
```

L9 ANSWER 6 OF 16 USPATFULL on STN
TI METHODS FOR USING **RESONANCE ENERGY TRANSFER**- BASED ASSAY OF **HIV**-1 ENVELOPE GYLCOPROTEIN-MEDIATED MEMBRANE FUSION, AND KITS FOR PRACTICING SAME

L9 ANSWER 7 OF 16 USPATFULL on STN
TI Helper virus-free herpesvirus amplicon particles and uses thereof

L9 ANSWER 8 OF 16 USPATFULL on STN
TI **HIV**-1 group O antigens and uses thereof

L9 ANSWER 9 OF 16 USPATFULL on STN
TI Method for preventing **HIV**-1 infection of **CD4**+ cells

L9 ANSWER 10 OF 16 USPATFULL on STN
TI Compositions and methods for inhibition of **HIV**-1 infection

L9 ANSWER 11 OF 16 USPATFULL on STN
TI Methods for characterizing the viral infectivity status of a host

L9 ANSWER 12 OF 16 USPATFULL on STN
TI Fluorescence **resonance energy transfer** screening assay for the identification of **HIV**-1 envelope glycoprotein-medicated cell

L9 ANSWER 13 OF 16 USPATFULL on STN
TI Compounds capable of inhibiting **HIV**-1 infection

L9 ANSWER 14 OF 16 USPATFULL on STN
TI Fluorescence **resonance energy transfer** screening assay for the identification of compounds that are capable of abrogating macrophage-tropic **HIV**-1 cell fusion

L9 ANSWER 15 OF 16 USPATFULL on STN
TI Method for preventing **HIV**-1 infection of **CD4**+ cells

L9 ANSWER 16 OF 16 USPATFULL on STN
TI Immunobiologically-active linear peptides and method of identification

=> d l9,cbib,ab,clm,1-15

L9 ANSWER 1 OF 16 USPATFULL on STN
2004:113689 Uses of a chemokine receptor for inhibiting **HIV**-1 infection.
Allaway, Graham P., Mohegan Lake, NY, UNITED STATES
Dragic, Tatjana, Hartsdale, NY, UNITED STATES
Litwin, Virginia M., Fayetteville, NY, UNITED STATES
Maddon, Paul J., Elmsford, NY, UNITED STATES
Moore, John P., New York, NY, UNITED STATES
Trkola, Alexandra, New York, NY, UNITED STATES
Progenics Pharmaceuticals, Inc. (U.S. corporation)Aaron Diamond AIDS Research Centre (ADARC) (U.S. corporation)
US 2004086528 A1 20040506
APPLICATION: US 2001-852238 A1 20010509 (9)
PRIORITY: US 1996-19941P 19960614 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a polypeptide comprising a fragment of a chemokine receptor capable of inhibiting **HIV**-1 infection. In an embodiment, the chemokine receptor is C--C CKR-5. In another embodiment, the fragment comprises at least one extracellular domain of the chemokine receptor C--C CKR-5. This invention further provides different uses of the chemokine receptor for inhibiting **HIV**-1 infection.
CLM What is claimed is:
1. A polypeptide having a sequence corresponding to the sequence of a portion of a chemokine receptor and capable of inhibiting the fusion of **HIV**-1 to **CD4**+ cells and thus of inhibiting **HIV**-1 infection of

the cells.

2. A polypeptide having a sequence corresponding to the sequence of a portion of the chemokine receptor, CCR5 and capable of inhibiting the fusion of **HIV**-1 to **CD4**+ cells and thus of inhibiting **HIV**-1 infection of the cells.

3. The polypeptide of claim 2 comprising amino acids having a sequence of at least one extracellular domain of CCR5.

4. The polypeptide of claim 3 wherein the extracellular domain is the second extracellular loop.

5. A pharmaceutical composition comprising an amount of the polypeptide of claim 1 effective to inhibit the fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

6. A polypeptide having a sequence corresponding to that of a portion of a **HIV**-1 envelope glycoprotein capable of specifically binding to the chemokine receptor CCR5.

7. The polypeptide of claim 6, wherein the glycoprotein is **gp120**.

8. A pharmaceutical composition comprising an effective amount of the polypeptide of claim 6 effective to inhibit the fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

9. An **antibody** or a portion of an **antibody** capable of binding to a chemokine receptor on a **CD4**+ cell and inhibiting **HIV**-1 infection of the cell.

10. A pharmaceutical composition comprising an amount of the **antibody** of claim 9 effective to inhibit **HIV**-1 infection of **CD4**+ cells and a pharmaceutically acceptable carrier.

11. A method of treating an **HIV**-1 infected subject which comprises administering to the subject the polypeptide of any of claims 1, 2, 3, 4, 6, or 7 in an amount effective to inhibit the fusion of **HIV**-1 to **CD4**+ cells of the subject and thus treat the subject.

12. A method of reducing the likelihood of a subject from becoming infected by **HIV**-1 which comprises administering to the subject the polypeptide of any of claims 1, 2, 3, 4, 6, or 7 in an amount effective to inhibit the fusion of **HIV**-1 to **CD4**+ cells of the subject and thus reduce the likelihood of **HIV**-1 infection.

13. A method for inhibiting **HIV**-1 infection of **CD4**+ cells which comprises contacting such **CD4**+ cells with a non-chemokine agent capable of binding to the chemokine receptor CCR5 in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited, thereby inhibiting **HIV**-1 infection of the cells.

14. The method of claim 13, wherein the non-chemokine agent is an oligopeptide.

15. The method of claim 13, wherein the non-chemokine agent is a polypeptide.

16. The method of claim 13, wherein the non-chemokine agent is a nonpeptidyl agent.

17. A non-chemokine agent capable of binding to the chemokine receptor CCR5 and inhibiting the fusion of **HIV**-1 to **CD4**+ cells.

18. A pharmaceutical composition comprising an amount of the non-chemokine agent capable of binding to the chemokine receptor CCR5

and inhibiting the fusion of **HIV**-1 to **CD4**+ cells effective to inhibit **HIV**-1 infection of **CD4**+ cells and a pharmaceutically acceptable carrier.

19. A molecule capable of binding to the chemokine receptor CCR5 and inhibiting fusion of **HIV**-1 to **CD4**+ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the **CD4**+ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor.

20. The molecule of claim 18, wherein the cell surface receptor is **CD4**.

21. The molecule of claim 18, wherein the ligand comprises an **antibody** or a portion of an **antibody**.

22. A molecule capable of binding to the chemokine receptor CCR5 and inhibiting fusion of **HIV**-1 to **CD4**+ cells comprising a non-chemokine agent linked to a compound capable of increasing the in vivo half-life of the non-chemokine agent.

23. The molecule of claim 21, wherein the compound is polyethylene glycol.

24. A pharmaceutical composition comprising an amount of the molecule of claim 19, 20, 21, 22 or 23 effective to inhibit fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

25. A method for reducing the likelihood of **HIV**-1 infection in a subject comprising administering the pharmaceutical composition of claim 19, 20, 21, 22 or 23 to the subject.

26. A method for treating **HIV**-1 infection in a subject comprising administering the pharmaceutical composition of claim 19, 20, 21, 22 or 23 to the subject.

27. A method for determining whether a non-chemokine agent is capable of inhibiting the fusion of **HIV**-1 to a **CD4**+, CCR5+ cell which comprises: (a) contacting the **CD4**+, CCR5+ cell, after it is labeled with a first dye, with a cell expressing an appropriate **HIV**-1 envelope glycoprotein on its surface, and labeled with a second dye, in the presence of an excess of the agent under conditions permitting fusion of the **CD4**+, CCR5+ cell to the cell expressing the **HIV**-1 envelope glycoprotein on its surface in the absence of an agent known to inhibit fusion of **HIV**-1 to **CD4**+, CCR5+ cells, the first and second dyes being selected so as to allow **resonance energy transfer** between the dyes; (b) exposing the product of step (a) to conditions which would result in **resonance energy transfer** if fusion has occurred; and (c) determining whether there is **resonance energy transfer**, the absence or reduction of transfer indicating that the agent is capable of inhibiting fusion of **HIV**-1 to **CD4**+ and CCR5+ cells.

28. The method of claim 27, wherein the agent is an oligopeptide, a polypeptide or a nonpeptidyl agent.

29. The method of claim 27, wherein the **CD4**+ cell is a PM1 cell.

30. The method of claim 27, wherein the cell expressing the **HIV**-1 envelope glycoprotein is a HeLa cell expressing **HIV**-1_{JR-FL} **gp120**/gp41.

31. A transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor CCR5.

32. The transgenic nonhuman animal of claim 31 further comprising an isolated DNA molecule encoding a sufficient portion of the **CD4** molecule to permit binding the **HIV**-1 envelope glycoprotein.

33. A transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor CCR5 and an isolated DNA molecule encoding fusin.

34. The transgenic nonhuman animal of claim 33 further comprising an isolated DNA molecule encoding a sufficient portion of the **CD4** molecule to permit binding the **HIV**-1 envelope glycoprotein.

35. A transformed cell which comprises an isolated nucleic acid molecule encoding the chemokine receptor CCR5.

36. An agent capable of inhibiting **HIV**-1 infection and capable of binding to a chemokine receptor without substantially affecting the said chemokine receptor's capability to bind to chemokines.

37. The agent of claim 36, wherein the said chemokine receptor is CCR5.

38. The agent of claim 36, wherein after the binding of the agent to the said chemokine receptor, a two fold higher concentration of the chemokine is required to achieve the degree of binding observed if the chemokine receptor had not been bound to the agent.

39. The agent of claim 36, wherein after the binding of the agent to the said chemokine receptor, a ten fold higher concentration of chemokine is required to achieve the degree of binding observed if the chemokine receptor had not been bound to the agent.

40. The agent of claim 36, wherein the agent is an oligopeptide, a nonpeptidyl agent or a polypeptide.

41. The agent of claim 40, wherein the polypeptide is an **antibody** or a portion of an **antibody**.

42. A pharmaceutical composition comprising an amount of the agent of claim 37, 38, 39, 40 or 41 effective to inhibit fusion of **HIV**-1 infection and a pharmaceutically acceptable carrier.

43. A method for inhibiting **HIV**-1 infection of **CD4**+ cells which comprises contacting such **CD4**+ cells with an agent capable of inhibiting **HIV**-1 infection and capable of binding to a chemokine receptor without substantially affecting the said chemokine receptor's capability to bind to chemokines.

44. A molecule capable of binding to the chemokine receptor CCR5 and inhibiting fusion of **HIV**-1 to **CD4**+ cells comprising the agent of claim 36 linked to a compound capable of increasing the in vivo half-life of the non-chemokine agent.

45. The molecule of claim 44, wherein the compound is polyethylene glycol.

46. A pharmaceutical composition comprising an amount of the molecule of claim 44 or 45 effective to inhibit fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

47. A method for reducing the likelihood of **HIV**-1 infection in a subject comprising administering the pharmaceutical composition of claim 42 or 46 to the subject.

48. A method for treating **HIV**-1 infection in a subject comprising administering the pharmaceutical composition of claim 42 or 46 to the subject.

L9 ANSWER 2 OF 16 USPATFULL on STN
2004:82318 Compositions and methods for inhibition of **HIV**-1 infection.
Olson, William C., Ossining, NY, UNITED STATES
Maddon, Paul J., Scarsdale, NY, UNITED STATES
Progenics Pharmaceuticals, Inc. (U.S. corporation)
US 2004062767 A1 20040401
APPLICATION: US 2003-681879 A1 20031009 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a composition which comprises an admixture of two compounds, wherein one compound retards attachment of **HIV**-1 to a **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell and the other compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate, wherein the relative mass ratio of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell. This invention also provides a method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of the above composition effective to inhibit **HIV**-1 infection of the **CD4**+ cell so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

CLM What is claimed is:

1. A composition which comprises an admixture of two compounds, wherein one compound retards attachment of **HIV**-1 to a **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell and the other compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate, wherein the relative mass ratio of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell.

2. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a **CD4**-based protein.

3. The composition of claim 2, wherein the **CD4**-based protein is a **CD4**-immunoglobulin fusion protein.

4. The composition of claim 3, wherein the **CD4**-immunoglobulin fusion protein is **CD4**-IgG2, wherein the **CD4**-IgG2 comprises two heavy chains and two lights chains, wherein the heavy chains are encoded by an expression vector designated **CD4**-IgG2HC-pRcCMV (ATCC Accession No. 75193) and the light chains are encoded by an expression vector designated **CD4**-kLC-pRcCMV (ATCC Accession No. 75194).

5. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a protein, the amino acid sequence of which comprises that of a protein found in **HIV**-1 as an-envelope glycoprotein.

6. The composition of claim 5, wherein the protein binds to an epitope of **CD4** on the surface of the **CD4**+ cell.

7. The composition of claim 6, wherein the envelope glycoprotein is selected from the group consisting of **gp120**, **gp160**, and gp140.

8. The composition of claim 1, wherein the compound which retards the attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell

is an **antibody** or portion of an **antibody**.

9. The composition of claim 8, wherein the **antibody** is a monoclonal **antibody**.

10. The composition of claim 9, wherein the monoclonal **antibody** is a human, humanized or chimeric **antibody**.

11. The composition of claim 8, wherein the portion of the **antibody** is a Fab fragment of the **antibody**.

12. The composition of claim 8, wherein the portion of the **antibody** comprises the variable domain of the **antibody**.

13. The composition of claim 8, wherein the portion of the **antibody** comprises a CDR portion of the **antibody**.

14. The composition of claim 9, wherein the monoclonal **antibody** is an IgG, IgM, IgD, IgA, or IgE monoclonal **antibody**.

15. The composition of claim 9, wherein the monoclonal **antibody** binds to an **HIV**-1 envelope glycoprotein.

16. The composition of claim 15, wherein the **HIV**-1 envelope glycoprotein is selected from the group consisting of **gp120** and **gp160**.

17. The composition of claim 16, wherein **HIV**-1 envelope glycoprotein is **gp120** and the monoclonal **antibody** which binds to **gp120** is IgG1b12 or F105.

18. The composition of claim 8, wherein the **antibody** binds to an epitope of **CD4** on the surface of the **CD4**+ cell.

19. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a peptide.

20. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a nonpeptidyl agent.

21. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is an **antibody**.

22. The composition of claim 21, wherein the **antibody** is a monoclonal **antibody**.

23. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a peptide.

24. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a fusion protein which comprises a peptide selected from the group consisting of T-20 (SEQ ID NO: 1), DP107 (SEQ ID NO: 2), N34 (SEQ ID NO: 3), C28 (SEQ ID NO: 4), and N34(L6)C28 (SEQ ID NO: 5).

25. The composition of claim 23, wherein the peptide is selected from the group consisting of T-20 (SEQ ID NO: 1), DP107 (SEQ ID NO: 2), N34 (SEQ ID NO: 3), C28 (SEQ ID NO: 4), and N34(L6)C28 (SEQ ID NO: 5).

26. The composition of claim 23, wherein the peptide is T-20 (SEQ ID NO: 1).

27. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a non-peptidyl agent.

28. The composition of claim 1, wherein the relative mass ratio of each such compound in the admixture ranges from about 25:1 to about 1:1.

29. The composition of claim 28, wherein the mass ratio is about 25:1.

30. The composition of claim 28, wherein the mass ratio is about 5:1.

31. The composition of claim 28, wherein the mass ratio is about 1:1.

32. The composition of claim 1, wherein the composition is admixed with a carrier.

33. The composition of claim 32, wherein the carrier is an aerosol, intravenous, oral or topical carrier.

34. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of the composition of claim 1 effective to inhibit **HIV**-1 infection of the **CD4**+ cell so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

35. The method of claim 34, wherein the **CD4**+ cell is present in a subject and the contacting is effected by administering the composition to the subject.

36. The method of claim 33, wherein the effective amount of the composition comprises from about 0.000001 mg/kg body weight to about 100 mg/kg body weight of the subject.

37. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of a compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell effective to inhibit **HIV**-1 infection of the **CD4**+ cell and an amount of a compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

38. The method of claim 37, wherein the **CD4**+ cell is present in a subject and the contacting is effected by administering the compounds to the subject.

39. The method of claim 38, wherein the compounds are administered to the subject simultaneously.

40. The method of claim 38, wherein the compounds are administered to the subject at different times.

41. The method of claim 38, wherein the compounds are administered to the subject by different routes of administration.

L9 ANSWER 3 OF 16 USPATFULL on STN
2004:13084 Potent oncolytic herpes simplex virus for cancer therapy.
Zhang, Xiaoliu, Houston, TX, UNITED STATES
Fu, Xinping, Zhuhai, CHINA
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US 2002-410024P 20020911 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to an oncolytic Herpes Simplex Virus having multiple cell membrane fusion mechanisms and preferably comprising a strict late viral promoter for effective conditional replication, such as in a malignant cell. In specific embodiments, the cell membrane fusion mechanisms are either from a mutant virus generated through random mutagenesis or through insertion of a fusogenic membrane glycoprotein, and in further specific embodiments the strict late viral promoter UL38p regulates expression of the glycoprotein.

CLM What is claimed is:

1. A composition, comprising: a vector comprising: a first cell membrane fusion-generating activity; and a second cell membrane fusion-generating activity.

2. The composition of claim 1, wherein said vector is a Herpes Simplex Virus vector.

3. The composition of claim 2, wherein the HSV vector is conditionally replicating.

4. The composition of claim 3, wherein conditionally replicating is defined as the vector comprising a strict late viral promoter.

5. The composition of claim 1, wherein the first cell membrane fusion-generating activity, the second cell membrane fusion-generating activity, or both comprise a mutation, said mutation conferring said cell membrane fusion-generating activity to the vector or a gene product encoded thereby.

6. The composition of claim 1, wherein the first cell membrane fusion-generating activity, the second cell membrane fusion-generating activity, or both comprise a nucleic acid sequence that encodes a fusogenic polypeptide.

7. The composition of claim 6, wherein the fusogenic polypeptide is further defined as a membrane glycoprotein.

8. The composition of claim 7, wherein the membrane glycoprotein is paramyxovirus F protein, **HIV gp160** protein, SIV **gp160** protein, retroviral Env protein, Ebola virus Gp, or the influenza virus haemagglutinin.

9. The composition of claim 7, wherein the glycoprotein is a membrane glycoprotein from gibbon ape leukemia virus (GALV).

10. The composition of claim 7, wherein the glycoprotein is a C-terminally truncated form of the gibbon ape leukemia virus envelope glycoprotein (GALV.fus).

11. The composition of claim 6, wherein the expression of the nucleic acid sequence is controlled by a strict late viral promoter.

12. The composition of claim 11, wherein the strict late viral promoter is the promoter of UL38 or Us11 of HSV.

13. The composition of claim 1, further comprising a pharmaceutically acceptable excipient.

14. A method of generating fusion between a first cell and a second cell, comprising the step of fusing the second cell membrane with the first cell membrane by introducing to the first cell a vector comprising a first cell membrane fusion-generating activity and a second cell

membrane fusion-generating activity.

15. The method of claim 14, wherein the first cell, second cell, or both first and second cells are malignant cells.

16. The method of claim 15, wherein the malignant cells are in a solid tumor.

17. The method of claim 15, wherein the malignant cells are in a human.

18. The method of claim 17, wherein the introducing step is further defined as delivering the vector to the human.

19. The method of claim 18, wherein the delivering step is further defined as systemically delivering the vector to the human.

20. The method of claim 19, wherein the systemic delivery to the human is further defined as intravenously delivering the vector to the human.

21. The method of claim 14, wherein the step is repeated with a plurality of cells.

22. The method of claim 14, wherein the vector is a conditionally replicating Herpes Simplex Virus vector.

23. The method of claim 14, wherein the first cell membrane fusion-generating activity, the second cell membrane fusion-generating activity, or both comprise a mutation, said mutation conferring said cell membrane fusion-generating activity to the vector or a gene product encoded thereby.

24. The method of claim 14, wherein the first cell membrane fusion-generating activity, the second cell membrane fusion-generating activity, or both comprise a nucleic acid sequence that encodes a fusogenic polypeptide.

25. The method of claim 24, wherein the expression of the nucleic acid sequence is regulated by a strict late viral promoter.

26. The method of claim 25, wherein the strict late viral promoter is the promoter of UL38 or Us11 of HSV.

27. The method of claim 17, wherein the method further comprises the step of providing enhanced tumor antigen presentation compared to in the absence of said vector.

28. The method of claim 27, wherein said enhanced tumor antigen presentation provides an improved antitumor immunity compared to in the absence of said enhanced tumor antigen presentation.

29. A method of destroying a malignant cell, comprising the step of introducing to the cell a vector comprising a first cell membrane fusion-generating activity; and a second cell membrane fusion-generating activity, wherein following said introduction the membrane of the malignant cell fuses with another cell membrane.

30. The method of claim 27, wherein the malignant cell is in a human.

31. The method of claim 28, wherein the introduction step is further defined as administering at least about 1×10^9 plaque forming units (pfu) of the vector to the human.

32. The method of claim 28, wherein the method further comprises administering an additional cancer therapy to the human.

33. The method of claim 30, wherein the additional cancer therapy is

chemotherapy, radiation, surgery, immunotherapy, gene therapy, or a combination thereof.

34. The method of claim 30, wherein the method further comprises the step of providing enhanced tumor antigen presentation compared to in the absence of said vector.

35. The method of claim 34, wherein said enhanced tumor antigen presentation provides an improved antitumor immunity compared to in the absence of said enhanced tumor antigen presentation.

36. A composition comprising an oncolytic virus, wherein the virus comprises a strict late viral promoter.

37. The composition of claim 32, wherein said virus is further defined as being tumor-specific.

38. A method of generating a cell membrane fusion-generating Herpes Simplex Virus vector comprising the steps of: introducing a mutation to a non-cell membrane fusion-generating Herpes Simplex Virus vector, said mutation conferring cell-membrane fusion-generating activity to the vector or a gene product encoded thereby; and incorporating into said vector a nucleic acid sequence encoding a cell membrane fusion-generating polypeptide.

39. A composition, comprising: a Herpes Simplex Virus vector comprising a mutation that confers to the vector or a gene product encoded thereby a cell membrane fusion-generating activity; and a nucleic acid sequence encoding GALV.fus.

40. A method of destroying a malignant cell comprising introducing to said cell a composition comprising an oncolytic virus, wherein the virus comprises a strict late viral promoter.

41. A mammalian cell comprising the composition of claim 1.

42. A mammalian cell comprising the composition of claim 35.

43. A vector, comprising a first cell membrane fusion-generating activity and a second cell membrane fusion-generating activity, wherein said vector is obtainable by a method comprising at least one of the following steps: generating a mutation in a nucleic acid sequence of the vector, wherein the mutation confers to the vector or a gene product encoded thereby the cell membrane fusion-generating activity; incorporating into the vector a nucleic acid sequence encoding a gene product comprising cell membrane fusion-generating activity; or both.

44. The vector of claim 39, wherein said incorporating step is further defined as: providing a first polynucleotide comprising a Herpes Simplex Virus genome, said Herpes Simplex Virus being non-infectious; providing a second polynucleotide comprising: the nucleic acid sequence encoding at least one gene product comprising cell membrane fusion-generating activity; and at least one nucleic acid sequence encoding a gene product comprising a functional packaging signal; and incorporating the nucleic acid sequence encoding a gene product comprising cell membrane fusion-generating activity and the nucleic acid sequence encoding a gene product comprising a functional packaging signal into the first polynucleotide, wherein said incorporating step generates an infectious Herpes Simplex Virus.

45. The vector of claim 40, wherein the incorporating the nucleic acid sequence encoding a gene product comprising cell membrane fusion-generating activity and the nucleic acid sequence encoding a gene product comprising a functional packaging signal into the first polynucleotide step is further defined as: mixing the first and second polynucleotides together to form a mixture; introducing the mixture to

a cell; and assaying for lysis of said cell.

46. The vector of claim 40, wherein the first polynucleotide is provided on a bacterial artificial chromosome.

47. The vector of claim 40, wherein the Herpes Simplex Virus of the first polynucleotide comprises: a deletion of γ34.5; a deletion of one or more copies of pac; or a combination thereof.

48. The vector of claim 40, wherein said infectious Herpes Simplex Virus is replication selective.

49. The vector of claim 40, wherein the second polynucleotide is provided on a plasmid.

50. The vector of claim 40, wherein the expression of the nucleic acid sequence encoding at least one gene product comprising cell membrane fusion-generating activity is regulated by CMV immediate early promoter.

51. A method of generating a vector comprising a first cell membrane fusion-generating activity and a second cell membrane fusion-generating activity, comprising at least one of the following steps: generating a mutation in a nucleic acid sequence of the vector, wherein the mutation confers to the vector or a gene product encoded thereby the cell membrane fusion-generating activity; incorporating into the vector a nucleic acid sequence encoding a gene product comprising cell membrane fusion-generating activity; or both.

52. The method of claim 47, wherein said incorporating step is further defined as: providing a first polynucleotide comprising a Herpes Simplex Virus genome, said Herpes Simplex Virus being non-infectious; providing a second polynucleotide comprising: the nucleic acid sequence encoding at least one gene product comprising cell membrane fusion-generating activity; and at least one nucleic acid sequence encoding a gene product comprising a functional packaging signal; and incorporating the nucleic acid sequence encoding a gene product comprising cell membrane fusion-generating activity and the nucleic acid sequence encoding a gene product comprising a functional packaging signal into the first polynucleotide, wherein said incorporating step generates an infectious Herpes Simplex Virus.

53. A vector obtained by the method of claim 47.

54. A method of increasing tumor antigen presentation in an individual, said individual comprising a malignant cell, comprising the step of providing to the individual a vector comprising a first cell membrane fusion-generating activity and a second cell membrane fusion-generating activity.

55. The method of claim 54, wherein said increased tumor antigen presentation provides an improved antitumor immunity in the individual compared to in the absence of said increased tumor antigen presentation.

L9 ANSWER 4 OF 16 USPATFULL on STN
2003:257708 **HIV**-1 group O antigens and uses thereof.
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Peeters, Martine, Saint Jean de Cuculles, FRANCE
Saman, Eric, Bornem, BELGIUM
Vanden Haesevelde, Marleen, Oudenaarde, BELGIUM
INNOGENETICS N.V. (non-U.S. corporation)
US 2003180759 A1 20030925
APPLICATION: US 2002-320786 A1 20021216 (10)
PRIORITY: EP 1997-870110 19970718
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The current invention relates to new **HIV**-1 group O antigens, nucleic acids encoding them, and the use of said antigens and/or nucleic acids as reagents in the diagnosis and prophylaxis of AIDS. It also relates to new **HIV**-1 group O strains comprising these antigens.

CLM What is claimed is:

1. Antigen derived from the **gp160** env precursor protein of a new **HIV**-1 group O strain comprising at least one amino acid sequence chosen from the following group of sequences:

VQQMKI,	(SEQ ID NO 53)
KIGPMSWYSMG,	(SEQ ID NO 54)
MGLEKN,	(SEQ ID NO 55)
IQQMKI,	(SEQ ID NO 56)
KIGPLAWYSMG,	(SEQ ID NO 57)
MGLERN,	(SEQ ID NO 58)
QSVQEIKI,	(SEQ ID NO 59)
KIGPMAWYSIG,	(SEQ ID NO 60)
IGIGTT,	(SEQ ID NO 61)
VQEIQT,	(SEQ ID NO 62)
QTGPMAWYSIH,	(SEQ ID NO 63)
IHLRTP,	(SEQ ID NO 64)
IQEIKI,	(SEQ ID NO 65)
KIGPMSWYSMG,	(SEQ ID NO 66)
MGIGQE,	(SEQ ID NO 67)
SVQELRI,	(SEQ ID NO 68)
RIGPMAWYSMT,	(SEQ ID NO 69)
MTLERD,	(SEQ ID NO 70)
SVQEIPI,	(SEQ ID NO 136) and/or at least

one amino acid sequence chosen from the following group of sequences:

RNQQLLNLWGCKGRLIC,	(SEQ ID NO 71)
CKGRLICYTSVQWNM,	(SEQ ID NO 72)
LWGCKGRIVC,	(SEQ ID NO 73)
SLWGCKGKLIC,	(SEQ ID NO 74)
CKGKSIC,	(SEQ ID NO 75)
CKGKIVC,	(SEQ ID NO 76)
CRGRQVC,	(SEQ ID NO 77)
CKGRLICYTSVH,	(SEQ ID NO 79)
CKGNLIC,	(SEQ ID NO 80)

CKGKMIC, (SEQ ID NO 81)

CKGRVVC, (SEQ ID NO 82) or a fragment of said antigen, said fragment consisting of at least 8, preferably 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 50 up to the maximum number of contiguous amino acids of the amino acid sequence of said antigen, with said fragment being characterized by the fact that it specifically reacts with **antibodies** raised against said antigen.

2. Antigen according to claim 1, characterized by an amino acid sequence comprising at least one of the following amino acid sequences:

(SEQ ID NO 83)
CERPGNNSIQQMKIGPLAWYSMGLERNKSSISRLAYC,

(SEQ ID NO 84)
CERPGNNSIQQMKIGPMAWYSMGLERNKSSISRLAYC,

(SEQ ID NO 85)
CERPGNQSVQEIKIGPMAWYSIGIGTTPANWSRIAYC,

(SEQ ID NO 86)
CERPGNQSVQEIKIGPMAWYSIGIGTTPTYNWSRIAYC,

(SEQ ID NO 87)
CVRPWNQTVQEIQTGPMAWYSIHLRTPLANLSRIAYC,

(SEQ ID NO 88)
CQRPGNLTIQEIKIGPMSWYSMGIGQEDHSKSRNAYC,

(SEQ ID NO 89)
CERPYYQSVQELRIGPMAWYSMTLERDRAGSDIRAAYC,

(SEQ ID NO 90)
CERPGNHTVQQMKIGPMSWYSMGLEKNNTSSRRAFC,

(SEQ ID NO 135)
CERTWNQSVQEIPIGPMAWYSMSVELDLNTTGSRSADC, and/or at least one amino acid sequence chosen from the following group of sequences:

DQQLLNLWGCKGRIVCYTSVKWN, (SEQ ID NO 91)

NQQLLNLWGCKGRLVCYTSVKWNK, (SEQ ID NO 92)

NQQLLNLWGCKGRLVCYTSVKWNN, (SEQ ID NO 138)

NQQRLNLWGCKGKMICYTSVPWN, (SEQ ID NO 93)

NQQLLNLWGCKGKSICYTSVKWN, (SEQ ID NO 94)

NQQLLNLWGCKGRLICYTSVQWN, (SEQ ID NO 95)

NQQRLNLWGCKGKMICYTSVKWN, (SEQ ID NO 96)

NQQLLNLWGCKGNLICYTSVKWN, (SEQ ID NO 97)

NQQLLNLWGCRGRQVCYTSVIWN, (SEQ ID NO 98)

SQQLLNLWGCKGRLICYTSVHWN, (SEQ ID NO 99)

NQQLLNLWGCKGRIVCYTSVKWN, (SEQ ID NO 100)

NQQLLNSWGCKGKIVCYTAVKWN, (SEQ ID NO 101)

NQQLLSLWGCKGKLICYTSVKWN, (SEQ ID NO 102)

NQQLLNLWGCKGRLVCYTSVQWN, (SEQ ID NO 137) or a fragment of said antigen, said fragment consisting of at least 8, preferably 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 50 up to the maximum number of contiguous amino acids of the amino acid sequence of said antigen, with said fragment being characterized by the fact that it specifically reacts with **antibodies** raised against said antigen.

3. Antigen according to any of claims 1 to 2, characterized by an amino acid sequence comprising at least one of the amino acid sequences represented by SEQ ID NO 2, SEQ ID NO 4, SEQ ID NO 6, SEQ ID NO 8, SEQ ID NO 10, SEQ ID NO 12, SEQ ID NO 14, SEQ ID NO 16, SEQ ID NO 18, SEQ ID NO 20, SEQ ID NO 22, SEQ ID NO 24, SEQ ID NO 26, SEQ ID NO 28, SEQ ID NO 30, SEQ ID NO 32, SEQ ID NO 34, SEQ ID NO 36, SEQ ID NO 38, SEQ ID NO 40 as shown in the alignment on FIG. 1, and/or at least one of the amino acid sequences represented by SEQ ID NO 42, SEQ ID NO 44, SEQ ID NO 46, SEQ ID NO 48, SEQ ID NO 50, or SEQ ID NO 52 as shown in the alignment on FIG. 2, and/or the amino acid sequence represented by SEQ ID NO 134, or a fragment of said antigen, said fragment consisting of at least 8, preferably 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 50 up to the maximum number of contiguous amino acids of any of the sequences represented by SEQ ID NO 2, SEQ ID NO 4, SEQ ID NO 6, SEQ ID NO 8, SEQ ID NO 10, SEQ ID NO 12, SEQ ID NO 14, SEQ ID NO 16, SEQ ID NO 18, SEQ ID NO 20, SEQ ID NO 22, SEQ ID NO 24, SEQ ID NO 26, SEQ ID NO 28, SEQ ID NO 30, SEQ ID NO 32, SEQ ID NO 34, SEQ ID NO 36, SEQ ID NO 38, SEQ ID NO 40, SEQ ID NO 42, SEQ ID NO 44, SEQ ID NO 46, SEQ ID NO 48, SEQ ID NO 50, SEQ ID NO 52, or SEQ ID NO 134 with said antigen fragment being characterized by the fact that it specifically reacts with **antibodies** raised against the antigen from which it is derived.

4. A polynucleic acid encoding an antigen according to any of claims 1 to 3, and more particularly a polynucleic acid comprising a nucleotide sequence chosen from the group of (I) a nucleotide sequence represented by SEQ ID NO 1, SEQ ID NO 3, SEQ ID NO 5, SEQ ID NO 7, SEQ ID NO 9, SEQ ID NO 11, SEQ ID NO 13, SEQ ID NO 15, SEQ ID NO 17, SEQ ID NO 19, SEQ ID NO 21, SEQ ID NO 23, SEQ ID NO 25, SEQ ID NO 27, SEQ ID NO 29, SEQ ID NO 31, SEQ ID NO 33, SEQ ID NO 35, SEQ ID NO 37, SEQ ID NO 39, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49, SEQ ID NO 51, SEQ ID NO 106 or (ii) a nucleotide sequence complementary to a sequence according to (I), or (iii) a nucleotide sequence showing at least 95%, preferably 96%, 97%, 98% and most preferably 99% homology to the fill length of a sequence according to (I), or (iv) a nucleotide sequence according to (I) whereby T is replaced by U, or (v) a nucleotide sequence according to (I) whereby at least one nucleotide is substituted by a nucleotide analogue.

5. A nucleic acid fragment consisting of a sequence of at least 15, preferably 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 up to 50 contiguous nucleotides of the sequence of a polynucleic acid according to claim 4, with said nucleic acid fragment being characterized by the fact that it selectively hybridizes to said polynucleic acid and/or selectively amplifies said polynucleic acid.

6. A virus strain belonging to **HIV**-1 group O, comprising in its genome a nucleic acid according to claim 4, and more particularly comprising in its genome the RNA equivalent of one of the DNA sequences represented by SEQ ID NO 1, SEQ ID NO 3, SEQ ID NO 5, SEQ ID NO 7, SEQ ID NO 9, SEQ ID NO 11, SEQ ID NO 13, SEQ ID NO 15, SEQ ID NO 17, SEQ ID NO 19, SEQ ID NO 21, SEQ ID NO 23, SEQ ID NO 25, SEQ ID NO 27, SEQ ID NO 29, SEQ ID NO 31, SEQ ID NO 33, SEQ ID NO 35, SEQ ID NO 37, SEQ ID NO 39, SEQ ID NO 106 and/or one of the DNA sequences represented by SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49, SEQ ID NO 51, and/or a variant sequence of the above-mentioned DNA sequences, said variant sequence showing at least 95% homology with the entire length of one of the above-mentioned sequences.

7. A virus strain according to claim 6, deposited at the ECACC on Jun. 13, 1997 under accession number V97061301, V97061302 or V97061303, or deposited at the ECACC on Jul. 13, 1998, under provisional accession number V98071301 or V98071302.

8. A polynucleic acid isolated from an **HIV**-1 group O strain according to any of claims 6 to 7.

9. An antigen isolated from an **HIV**-1 group O strain according to any of claims 6 to 7.

10. An **antibody**, preferably a monoclonal **antibody**, raised against an antigen or antigen fragment according to any of claims 1 to 3, or claim 9, with said **antibody** recognizing specifically the antigen or the antigen fragment to which it has been raised.

11. A method for detecting the presence of an **HIV**-1 infection, said method comprising the detection of **antibodies** against **HIV**-1, including **HIV**-1 group O, using an antigen or antigen fragment according to any of claims 1 to 3, or claim 9, and/or the detection of viral antigen originating from **HIV**-1, including **HIV**-1 group O, using an **antibody** according to claim 10, and/or the detection of viral nucleic acid originating from **HIV**-1, including **HIV**-1 group O, using a nucleic acid or nucleic acid fragment according to claims 4 or 5, or claim 8, in a biological sample.

12. A kit for the detection of the presence of an **HIV**-1 infection, comprising at least one of the antigens or antigen fragments according to any of claims 1 to 3, or claim 9, and/or at least one of the nucleic acids or nucleic acid fragments according to claim 4 or 5, or claim 8 and/or an **antibody** according to claim 10.

13. A vaccine composition which provides protective immunity against an **HIV**-1 infection, including an **HIV**-1 type O infection, comprising as an active principle at least one antigen or antigen fragment according to claims 1 to 3, or 9, or at least one nucleic acid according to claims 4 to 5, or 8 or a virus like particle (VLP) comprising at least one antigen or antigen fragment according to claims 1 to 3, or 9, or an attenuated form of at least one of the **HIV**-1 type O strains according to claims 6 to 7, said active principle being combined with a pharmaceutically acceptable carrier.

L9 ANSWER 5 OF 16 USPATFULL on STN
2003:119700 Compositions and methods for inhibition of **hiv**-1 infection.
Olson, William C., Ossining, NY, UNITED STATES
Maddon, Paul J., Scarsdale, NY, UNITED STATES
US 2003082185 A1 20030501
APPLICATION: US 2000-493346 A1 20000128 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a composition which comprises an admixture of two compounds, wherein one compound retards attachment of **HIV**-1 to a **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell and the other compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate, wherein the relative mass ratio of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell. This invention also provides a method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of the above composition effective to inhibit **HIV**-1 infection of the **CD4**+ cell so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.
CLM What is claimed is:

1. A composition which comprises an admixture of two compounds, wherein one compound retards attachment of **HIV**-1 to a **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell and the other compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate, wherein the relative mass ratio of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell.

2. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a **CD4**-based protein.

3. The composition of claim 2, wherein the **CD4**-based protein is a **CD4**-immunoglobulin fusion protein.

4. The composition of claim 3, wherein the **CD4**-immunoglobulin fusion protein is **CD4**-IgG2, wherein the **CD4**-IgG2 comprises two heavy chains and two lights chains, wherein the heavy chains are encoded by an expression vector designated **CD4**-IgG2HC-pRcCMV (ATCC Accession No. 75193) and the light chains are encoded by an expression vector designated **CD4**-kLC-pRcCMV (ATCC Accession No. 75194).

5. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a protein, the amino acid sequence of which comprises that of a protein found in **HIV**-1 as an envelope glycoprotein.

6. The composition of claim 5, wherein the protein binds to an epitope of **CD4** on the surface of the **CD4**+ cell.

7. The composition of claim 6, wherein the envelope glycoprotein is selected from the group consisting of **gp120**, **gp160**, and gp140.

8. The composition of claim 1, wherein the compound which retards the attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is an **antibody** or portion of an **antibody**.

9. The composition of claim 8, wherein the **antibody** is a monoclonal **antibody**.

10. The composition of claim 9, wherein the monoclonal **antibody** is a human, humanized or chimeric **antibody**.

11. The composition of claim 8, wherein the portion of the **antibody** is a Fab fragment of the **antibody**.

12. The composition of claim 8, wherein the portion of the **antibody** comprises the variable domain of the **antibody**.

13. The composition of claim 8, wherein the portion of the **antibody** comprises a CDR portion of the **antibody**.

14. The composition of claim 9, wherein the monoclonal **antibody** is an IgG, IgM, IgD, IgA, or IgE monoclonal **antibody**.

15. The composition of claim 9, wherein the monoclonal **antibody** binds to an **HIV**-1 envelope glycoprotein.

16. The composition of claim 15, wherein the **HIV**-1 envelope glycoprotein is selected from the group consisting of **gp120** and **gp160**.

17. The composition of claim 16, wherein **HIV**-1 envelope glycoprotein is **gp120** and the monoclonal **antibody** which binds to **gp120** is IgG1b12 or F105.

18. The composition of claim 8, wherein the **antibody** binds to an epitope of **CD4** on the surface of the **CD4**+ cell.

19. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a peptide.

20. The composition of claim 1, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a nonpeptidyl agent.

21. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is an **antibody**.

22. The composition of claim 21, wherein the **antibody** is a monoclonal **antibody**.

23. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a peptide.

24. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a fusion protein which comprises a peptide selected from the group consisting of T-20 (SEQ ID NO: 1), DP107 (SEQ ID NO: 2), N34 (SEQ ID NO: 3), C28 (SEQ ID NO: 4), and N34(L6)C28 (SEQ ID NO: 5).

25. The composition of claim 23, wherein the peptide is selected from the group consisting of T-20 (SEQ ID NO: 1), DP107 (SEQ ID NO: 2), N34 (SEQ ID NO: 3), C28 (SEQ ID NO: 4), and N34(L6)C28 (SEQ ID NO: 5).

26. The composition of claim 23, wherein the peptide is T-20 (SEQ ID NO: 1).

27. The composition of claim 1, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a non-peptidyl agent.

28. The composition of claim 1, wherein the relative mass ratio of each such compound in the admixture ranges from about 25:1 to about 1:1.

29. The composition of claim 28, wherein the mass ratio is about 25:1

30. The composition of claim 28, wherein the mass ratio is about 5:1.

31. The composition of claim 28, wherein the mass ratio is about 1:1.

32. The composition of claim 1, wherein the composition is admixed with a carrier.

33. The composition of claim 32, wherein the carrier is an aerosol, intravenous, oral or topical carrier.

34. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of the composition

of claim 1 effective to inhibit **HIV**-1 infection of the **CD4+** cell so as to thereby inhibit **HIV**-1 infection of the **CD4+** cell.

35. The method of claim 34, wherein the **CD4+** cell is present in a subject and the contacting is effected by administering the composition to the subject.

36. The method of claim 33, wherein the effective amount of the composition comprises from about 0.000001 mg/kg body weight to about 100 mg/kg body weight of the subject.

37. A method of inhibiting **HIV**-1 infection of a **CD4+** cell which comprises contacting the **CD4+** cell with an amount of a compound which retards attachment of **HIV**-1 to the **CD4+** cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4+** cell effective to inhibit **HIV**-1 infection of the **CD4+** cell and an amount of a compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4+** cell by binding noncovalently to an epitope on a gp41 fusion intermediate so as to thereby inhibit **HIV**-1 infection of the **CD4+** cell.

38. The method of claim 37, wherein the **CD4+** cell is present in a subject and the contacting is effected by administering the compounds to the subject.

39. The method of claim 38, wherein the compounds are administered to the subject simultaneously.

40. The method of claim 38, wherein the compounds are administered to the subject at different times.

41. The method of claim 38, wherein the compounds are administered to the subject by different routes of administration.

L9 ANSWER 6 OF 16 USPATFULL on STN
2003:64649 METHODS FOR USING **RESONANCE ENERGY TRANSFER**- BASED ASSAY OF **HIV**-1 ENVELOPE GYLCOPROTEIN-MEDIATED MEMBRANE FUSION, AND KITS FOR PRACTICING SAME.
ALLAWAY, GRAHAM P., MORETON MERSEYSIDE, UNITED KINGDOM
LITWIN, VIRGINIA M., FAYETTEVILLE, NY, UNITED STATES
MADDON, PAUL J., ELMSFORD, NY, UNITED STATES
US 2003044770 A1 20030306
APPLICATION: US 1999-412284 A1 19991005 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides: agents determined to be capable of specifically inhibiting the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4+** cell, but not a T cell-tropic isolate of **HIV**-1 to a **CD4+** cell; and agents determined to be capable of specifically inhibiting the fusion of a T cell-tropic isolate of **HIV**-1 to a **CD4+** cell, but not a macrophage-tropic primary isolate of **HIV**-1 to a **CD4+** cell. This invention also provides: agents capable of specifically inhibiting the fusion of a macrophage tropic primary isolate of **HIV**-1 with a CD+ cell susceptible to infection by a macrophage-tropic primary isolate of **HIV**-1; and agents capable of specifically inhibiting the fusion of a T cell-tropic isolate of **HIV**-1 with a **CD4+** cell susceptible to infection by a T cell-tropic isolate of HIV-1. The agents include but are not limited to **antibodies**. This invention further provides: methods of inhibiting fusion of a macrophage-tropic primary isolate of **HIV**-1 with a CD+ cell susceptible to infection by a macrophage-tropic primary isolate of **HIV**-1 which comprises contacting the **CD4+** cell with an amount of an agent capable of specifically inhibiting such fusion so as to thereby inhibit such fusion; and methods of inhibiting fusion of a T cell-tropic isolate of **HIV**-1 with a **CD4+** cell susceptible to

infection by a T cell-tropic isolate of **HIV**-1 which comprises contacting the **CD4**+ cell with an amount of an agent capable of specifically inhibiting such fusion so as to thereby inhibit such fusion.

CLM What is claimed is:

1. A method for determining whether an agent: is capable of inhibiting the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4**+ cell which comprises: (a) contacting (i) an appropriate **CD4**+ cell, which is labeled with a first dye, with (ii) a cell expressing the **HIV**-1 envelope glycoprotein of the macrophage-tropic primary isolate of **HIV**-1 on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the **CD4**+ cell to the cell expressing the **HIV**-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow **resonance energy transfer** between the dyes; (b) exposing the product of step (a) to conditions which would result in **resonance energy transfer** if fusion has occurred; and (c) determining whether there is a reduction of **resonance energy transfer**, when compared with the **resonance energy transfer** in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of **HIV**-1 to **CD4**+ cells.

2. The method of claim 1, wherein the **CD4**+ cell is a PM1 cell, a primary human T lymphocyte, or a primary human macrophage.

3. The method of claim 1, wherein the **HIV**-1 envelope glycoprotein+ cell is an **HIV**-1_{JR-FL} **gp120**/gp41 HeLa cell.

4. The method of claim 1 wherein the agent is not previously known.

5. An agent determined to be capable of inhibiting the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4**+ cell using the method of claim 1.

6. A therapeutic agent capable of inhibiting the fusion of an **HIV**-1 envelope glycoprotein+ cell with an appropriate **CD4**+ cell using the method of claim 1.

L9 ANSWER 7 OF 16 USPATFULL on STN

2003:37677 Helper virus-free herpesvirus amplicon particles and uses thereof.

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Bowers, William J., Webster, NY, UNITED STATES
Frelinger, John G., Pittsford, NY, UNITED STATES
Willis, Richard A., Denver, CO, UNITED STATES
Evans, Thomas D., Davis, CA, UNITED STATES
Dewhurst, Stephen, Rochester, NY, UNITED STATES
Tolba, Khaled A., Rochester, NY, UNITED STATES
Rosenblatt, Joseph D., Ft. Lauderdale, FL, UNITED STATES

US 2003027322 A1 20030206
APPLICATION: US 2001-997848 A1 20011129 (9)
PRIORITY: US 2000-253858P 20001129 (60)
US 2000-250079P 20001130 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention features new helper virus-free methods for making herpesvirus amplicon particles that can be used in immunotherapies, including those for treating any number of infectious diseases and cancers (including chronic lymphocytic leukemia, other cancers in which blood cells become malignant, lymphomas (e.g. Hodgkin's lymphoma or non-Hodgkin's type lymphomas). Described herein are methods of making helper virus-free HSV amplicon particles; cells that contain those particles (e.g., packaging cell lines or patients'cells, infected in vivo or ex vivo); particles produced according to those methods; and methods of treating a patient with an hf-HSV particle made according to

those methods.

CLM What is claimed is:

1. A method of generating a herpesvirus amplicon particle, the method comprising providing a cell that has been stably transfected with a nucleic acid sequence that encodes an accessory protein; and transfecting the cell with (a) one or more packaging vectors that, individually or collectively, encode one or more HSV structural proteins but do not encode a functional herpesvirus cleavage/packaging site and (b) an amplicon plasmid comprising a sequence that encodes a functional herpesvirus cleavage/packaging site and a herpesvirus origin of DNA replication.

2. A method of generating a herpesvirus amplicon particle, the method comprising transfecting a cell with (a) one or more packaging vectors that, individually or collectively, encode one or more HSV structural proteins but do not encode a functional herpesvirus cleavage/packaging site; (b) an amplicon plasmid comprising a sequence that encodes a functional herpesvirus cleavage/packaging site, a herpesvirus origin of DNA replication, and a sequence that encodes an immunomodulatory protein, a tumor-specific antigen, or an antigen of an infectious agent; and (c) a nucleic acid sequence that encodes an accessory protein.

3. The method of claim 1 or claim 2, wherein the herpesvirus is an alpha herpesvirus or an Epstein-Barr virus.

4. The method of claim 3, wherein the alpha herpesvirus is a Varicella-Zoster virus, a pseudorabies virus, or a herpes simplex virus.

5. The method of claim 1 or claim 2, wherein the accessory protein inhibits the expression of a gene in the cell.

6. The method of claim 5, wherein the accessory protein is a virion host shutoff protein.

7. The method of claim 6, wherein the virion host shutoff protein is an HSV-1 virion host shutoff protein, an HSV-2 virion host shutoff protein, an HSV-3 virion host shutoff protein, bovine herpesvirus 1 virion host shutoff protein, bovine herpesvirus 1.1 virion host shutoff protein, gallid herpesvirus 1 virion host shutoff protein, gallid herpesvirus 2 virion host shutoff protein, suid herpesvirus 1 virion host shutoff protein, baboon herpesvirus 2 virion host shutoff protein, pseudorabies virus virion host shutoff protein, cercopithecine herpesvirus 7 virion host shutoff protein, meleagrid herpesvirus 1 virion host shutoff protein, equine herpesvirus 1 virion host shutoff protein, or equine herpesvirus 4 virion host shutoff protein.

8. The method of claim 6, wherein the virion host shutoff protein is operatively coupled to its native transcriptional control elements.

9. The method of claim 1 or claim 2, wherein the cell is further transfected with a sequence encoding a VP16 protein, wherein the VP16 protein is transiently or stably expressed.

10. The method of claim 9, wherein the VP16 protein is HSV1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, or equine herpesvirus 4 VP16.

11. The method of claim 1 or claim 2, wherein the one or more packaging vectors comprises a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome, a human artificial chromosome, or an F element plasmid.

12. The method of claim 1 or claim 2, wherein the one or more packaging vectors comprises a set of cosmids comprising cos 6Δa, cos 28, cos 14, cos 56, and cos 48Δa.

13. The method of claim 1 or claim 2, wherein the one or more packaging vectors, individually or collectively, express the structural herpesvirus proteins.

14. The method of claim 1 or claim 2, wherein the herpesvirus origin of DNA replication is not present in the one or more packaging vectors.

15. The method of claim 1, wherein the amplicon plasmid further comprises a sequence encoding a therapeutic agent.

16. The method of claim 15, wherein the therapeutic agent is a protein or an RNA molecule.

17. The method of claim 16, wherein the RNA molecule is an antisense RNA molecule, RNAi, or a ribozyme.

18. The method of claim 16, wherein the protein is a receptor, a signaling molecule, a transcription factor, a growth factor, an apoptosis inhibitor, an apoptosis promoter, a DNA replication factor, an enzyme, a structural protein, a neural protein, or a histone.

19. The method of claim 16, wherein the protein is an immunomodulatory protein, a tumor-specific antigen, or an antigen of an infectious agent.

20. The method of claim 19, wherein the immunomodulatory protein is a cytokine or a costimulatory molecule.

21. The method of claim 20, wherein the cytokine is an interleukin, an interferon, or a chemokine.

22. The method of claim 20, wherein the costimulatory molecule is a B7 molecule or **CD40L**.

23. The method of claim 19, wherein the tumor-specific antigen is a prostate specific antigen.

24. The method of claim 19, wherein the infectious agent is a virus.

25. The method of claim 24, wherein the virus is a **human immunodeficiency virus**.

26. The method of claim 19, wherein the antigen of an infectious agent is **gp120**.

27. The method of claim 19, wherein the infectious agent is a bacterium or parasite.

28. The method of claim 2, wherein the immunomodulatory protein is a cytokine or a costimulatory molecule.

29. The method of claim 28, wherein the cytokine is an interleukin, an interferon, or a chemokine.

30. The method of claim 28, wherein the costimulatory molecule is a B7 molecule or **CD40L**.

31. The method of claim 2, wherein the tumor-specific antigen is a prostate specific antigen.

32. The method of claim 2, wherein the infectious agent is a virus.

33. The method of claim 32, wherein the virus is a **human immunodeficiency virus**.

34. The method of claim 2, wherein the antigen of an infectious agent is

gp120.

35. The method of claim 2, wherein the infectious agent is a bacterium or parasite.

36. The method of claim 1 or claim 2, wherein the amplicon plasmid further comprises a promoter.

37. A cell transfected by the method of claim 1 or transduced by a herpesvirus amplicon particle made by the method of claim 1.

38. The cell of claim 37, wherein the cell is a neuron, a blood cell, a hepatocyte, a keratinocyte, a melanocyte, a neuron, a glial cell, an endocrine cell, an epithelial cell, a muscle cell, a prostate cell, or a testicular cell.

39. A cell transfected by the method of claim 2 or transduced by a herpesvirus amplicon particle made by the method of claim 2.

40. The cell of claim 39, wherein the cell is a neuron, a blood cell, a hepatocyte, a keratinocyte, a melanocyte, a neuron, a glial cell, an endocrine cell, an epithelial cell, a muscle cell, a prostate cell, or a testicular cell.

41. The cell of claim 39, wherein the cell is a malignant cell.

42. The cell of claim 39, wherein the cell is infected with an infectious agent.

43. The cell of claim 42, wherein the infectious agent is a virus, a bacterium, or a parasite.

44. The cell of claim 43, wherein the virus is an immunodeficiency virus.

45. A herpesvirus amplicon particle made by the method of claim 1.

46. The herpesvirus amplicon particle of claim 45, wherein the herpesvirus is an alpha herpesvirus or an Epstein-Barr virus.

47. The herpesvirus amplicon particle of claim 46, wherein the alpha herpesvirus is a Varicella-Zoster virus, a pseudorabies virus, or a herpes simplex virus.

48. The herpesvirus amplicon particle of claim 47, wherein the herpes simplex virus is a type 1 or a type 2 herpes simplex virus.

49. A herpesvirus amplicon particle made by the method of claim 2.

50. The herpesvirus amplicon particle of claim 49, wherein the herpesvirus is an alpha herpesvirus or an Epstein-Barr virus.

51. The herpesvirus amplicon particle of claim 50, wherein the alpha herpesvirus is a Varicella-Zoster virus, a pseudorabies virus, or a herpes simplex virus.

52. The herpesvirus amplicon particle of claim 51, wherein the herpes simplex virus is a type 1 or a type 2 herpes simplex virus.

53. A method of treating a patient who has cancer, or who may develop cancer, the method comprising administering to the patient an HSV amplicon particle of claim 19, wherein the protein is an immunomodulatory protein or a tumor-specific antigen, or an HSV amplicon particle made by the method of claim 2, wherein the protein is an immunomodulatory protein or a tumor-specific antigen.

54. A method of treating a patient who has cancer, or who may develop cancer, the method comprising administering to the patient the cell of claim 37, wherein the amplicon plasmid further encodes an immunomodulatory protein or a tumor-specific antigen, or the cell of claim tumor-specific antigen, or an HSV amplicon particle made by the method of claim 39, wherein the protein is an immunomodulatory protein or a tumor-specific antigen.

55. A method of treating a patient who has a disease caused by an infectious agent, or who may contract a disease caused by an infectious agent, the method comprising administering to the patient the herpesvirus amplicon particle of claim 45, wherein the amplicon plasmid further comprises a sequence that encodes an antigen of the infectious agent, or the cell of claim 39, wherein the amplicon plasmid comprises a sequence that encodes an antigen of an infectious agent.

L9 ANSWER 8 OF 16 USPATFULL on STN
2003:26241 **HIV**-1 group O antigens and uses thereof.
DeLaporte, Eric, Saint Jean de Cuculles, FRANCE
Peeters, Martine, Saint Jean de Cuculles, FRANCE
Saman, Eric, Bornem, BELGIUM
Vanden Haesevelde, Marleen, Oudenaarde, BELGIUM
Innogenetics, N.V., BELGIUM (non-U.S. corporation)
US 6511801 B1 20030128
WO 9904011 19990128
APPLICATION: US 2000-462917 20000403 (9)
WO 1998-EP4522 19980720
PRIORITY: EP 1997-870110 19970718
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The claimed invention relates to an **HIV**-1 group O envelope antigen comprising SEQ ID NO: 100, and the use of said antigen as a reagent in the diagnosis of **HIV**-1 group O infection, and a kit therefore.
CLM What is claimed is:
1. An isolated antigen from the **HIV**-1 group O strain **gp160** env precursor protein comprising the amino acid sequence of SEQ ID NO:100.

2. A method for detecting anti-**HIV**-1 **antibodies** in a sample comprising: a) contacting the sample with an isolated antigen from the **HIV**-1 group O strain **gp160** env precursor protein comprising the amino acid sequence of SEQ ID NO:100, b) allowing the isolated antigen and anti-**HIV** **antibodies** to interact, and c) detecting the interaction between the antigen and the anti-**HIV** **antibodies**.

3. A kit for detecting **HIV**-1 **antibodies** comprising an isolated antigen from the **HIV**-1 group O strain **gp160** env precursor protein comprising the amino acid sequence of SEQ ID NO:100.

4. An immunogenic composition comprising: a) an isolated antigen from the **HIV**-1 group O strain **gp160** env precursor protein which comprises the amino acid sequence of SEQ ID NO:100; and b) a pharmaceutically acceptable carrier.

L9 ANSWER 9 OF 16 USPATFULL on STN
2002:279995 Method for preventing **HIV**-1 infection of **CD4**+ cells.
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Litwin, Virginia M., Fayetteville, NY, UNITED STATES
Maddon, Paul J., Elmsford, NY, UNITED STATES
Olson, William C., Ossining, NY, UNITED STATES
Progenics Pharmaceuticals, Inc. (U.S. corporation)
US 2002155429 A1 20021024
APPLICATION: US 2001-888938 A1 20010625 (9)
PRIORITY: US 1996-19715P 19960614 (60)
US 1996-14532P 19960402 (60)

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods for inhibiting fusion of **HIV**-1 to **CD4**+ cells which comprise contacting **CD4**+ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited. This invention also provides methods for inhibiting **HIV**-1 infection of **CD4**+ cells which comprise contacting **CD4**+ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited, thereby inhibiting the **HIV**-1 infection. This invention provides non-chemokine agents capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells. This invention also provides pharmaceutical compositions comprising an amount of the non-chemokine agent capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells effective to prevent fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

CLM What is claimed is:

1. A method for inhibiting fusion of **HIV**-1 to **CD4**+ cells which comprises contacting **CD4**+ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited.

2. A method for inhibiting **HIV**-1 infection of **CD4**+ cells which comprises contacting **CD4**+ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited, thereby inhibiting **HIV**-1 infection.

3. The method of claim 1 or 2, wherein the non-chemokine agent is an oligopeptide.

4. The method of claim 1 or 2, wherein the non-chemokine agent is a polypeptide.

5. The method of claim 1 or 2, wherein the non-chemokine agent is an **antibody** or a portion of an **antibody**.

6. The method of claim 1 or 2, wherein the non-chemokine agent is a nonpeptidyl agent.

7. A non-chemokine agent capable of binding to a chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells.

8. The non-chemokine agent of claim 7, wherein the non-chemokine agent is a oligopeptide.

9. The non-chemokine agent of claim 7, wherein the non-chemokine agent is a nonpeptidyl agent.

10. The non-chemokine agent of claim 7, wherein the non-chemokine agent is a polypeptide.

11. The non-chemokine agent of claim 10, wherein the polypeptide is an **antibody** or a portion of an **antibody**.

12. The non-chemokine agent of claim 10, wherein the polypeptide comprises amino acid sequence as set forth in SEQ ID NO:5.

13. The non-chemokine agent of claim 10, wherein the polypeptide comprises the MIP-1β sequence with the deletion of the first seven N-terminal amino acids of said sequence.

14. The non-chemokine agent of claim 10, wherein the polypeptide

comprises the MIP-1β sequence with the deletion of the first eight N-terminal amino acids of said sequence.

15. The non-chemokine agent of claim 10, wherein the polypeptide comprises the MIP-1β sequence with the deletion of the first nine N-terminal amino acids of said sequence.

16. The non-chemokine agent of claim 10, wherein the polypeptide comprises the MIP-1β sequence with the deletion of the first ten N-terminal amino acids of said sequence.

17. The non-chemokine agent of claim 10, wherein the polypeptide comprises the MIP-1β sequence with the N-terminal sequence modified by addition of an amino acid or oligopeptide.

18. The non-chemokine agent of claim 10, wherein the polypeptide comprises the MIP-1β sequence with the N-terminal sequence modified by removing the N-terminal alanine and replacing it by serine or threonine and an additional amino acid or oligopeptide or nonpeptidyl moiety.

19. The non-chemokine agent of claim 17 or 18, wherein the additional amino acid is methionine.

20. An agent capable of binding to CXCR4 and inhibiting **HIV**-1 infection.

21. The agent of claim 20, wherein the agent is an oligopeptide.

22. The agent of claim 20, wherein the agent is a polypeptide.

23. The non-chemokine agent of claim 22, wherein the polypeptide comprises the SDF-1 sequence with the deletion of the first six N-terminal amino acids of said sequence.

24. The non-chemokine agent of claim 22, wherein the polypeptide comprises the SDF-1 sequence with the deletion of the first seven N-terminal amino acids of said sequence.

25. The non-chemokine agent of claim 22, wherein the polypeptide comprises the SDF-1 sequence with the deletion of the first eight N-terminal amino acids of said sequence.

26. The non-chemokine agent of claim 22, wherein the polypeptide comprises the SDF-1 sequence with the deletion of the first nine N-terminal amino acids of said sequence.

27. The non-chemokine agent of claim 22, wherein the N-terminal glycine of SDF-1 is replaced by serine and derivatized with biotin.

28. The non-chemokine agent of claim 22, wherein the N-terminal glycine of SDF-1 is replaced by serine and derivatized with methionine.

29. The non-chemokine agent of claim 22, wherein the N-terminus of SDF-1 is modified by the addition of a methionine before the terminal glycine.

30. The agent of claim 22, wherein the agent is an **antibody** or a portion of an **antibody**.

31. The agent of claim 20, wherein the agent is a non-peptidyl agent.

32. A pharmaceutical composition comprising an amount of the non-chemokine agent of claim 7 effective to inhibit fusion of **HIV**-1 to **CD4+** cells and a pharmaceutically acceptable carrier.

33. A pharmaceutical composition comprising an amount of the

non-chemokine agent of claim 20 effective to inhibit fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

34. A composition of matter capable of binding to a chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the **CD4**+ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not inhibit the binding of the ligand to the other receptor.

35. The composition of matter of claim 34, wherein the cell surface receptor is **CD4**.

36. The composition of matter of claim 34, wherein the ligand comprises an **antibody** or a portion of an **antibody**.

37. A pharmaceutical composition comprising an amount of the composition of matter of claim 34 effective to inhibit fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

38. A composition of matter capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells comprising a non-chemokine agent linked to a compound capable of increasing the in vivo half-life of the non-chemokine agent.

39. The composition of matter of claim 38, wherein the compound is polyethylene glycol.

40. A pharmaceutical composition comprising an amount of the composition of claim 38 effective to inhibit fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

41. A method for reducing the likelihood of **HIV**-1 infection in a subject comprising administering the pharmaceutical composition of claim 32, 33, 37 or 40 to the subject.

42. A method for treating **HIV**-1 infection in a subject comprising administering the pharmaceutical composition of claim 32, 33, 39 or 40 to the subject.

43. A method for determining whether a non-chemokine agent is capable of inhibiting the fusion of **HIV**-1 to a **CD4**+ cell which comprises: (a) contacting (i) a **CD4**+ cell, which is labeled with a first dye, with (ii) a cell expressing the **HIV**-1 envelope glycoprotein on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the **CD4**+ cell to the cell expressing the **HIV**-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow **resonance energy transfer** between the dyes; (b) exposing the product of step (a) to conditions which would result in **resonance energy transfer** if fusion has occurred; and (c) determining whether there is a reduction of **resonance energy transfer**, when compared with the **resonance energy transfer** in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of **HIV**-1 to **CD4**+ cells.

44. The method of claim 43, wherein the agent is an oligopeptide.

45. The method of claim 43, wherein the agent is a polypeptide.

46. The method of claim 43, wherein the agent is an **antibody** or a portion of an **antibody**.

47. The method of claim 43, wherein the agent is a nonpeptidyl agent.

48. The method of claim 43, wherein the **CD4+** cell is a PM1 cell.

49. The method of claim 43, wherein the cell expressing the **HIV**-1 envelope glycoprotein is a HeLa cell expressing **HIV**-$1_{JR\text{-}FL}$ **gp120**/gp41.

50. The method of claim 43, wherein the cell expressing the **HIV**-1 envelope glycoprotein is a HeLa cell expressing **HIV**-1_{LAI} **gp120**/gp41.

L9 ANSWER 10 OF 16 USPATFULL on STN
2002:198280 Compositions and methods for inhibition of **HIV**-1 infection.
Olson, William C., Ossining, NY, UNITED STATES
Maddon, Paul J., Scarsdale, NY, UNITED STATES
US 2002106374 A1 20020808
APPLICATION: US 2001-912824 A1 20010725 (9)
PRIORITY: US 2001-266738P 20010206 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a composition which comprises an admixture of three compounds, wherein: (a) one compound is an **antibody** which binds to a CCR5 receptor; (b) one compound retards attachment of **HIV**-1 to a **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell; and (c) one compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate; wherein the relative mass ratio of any two of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell. This invention also provides a method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of the composition of the subject invention effective to inhibit **HIV**-1 infection of the **CD4**+ cell so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

CLM What is claimed is:

1. A composition which comprises an admixture of two compounds, wherein: (a) one compound is an **antibody** or portion thereof which binds to a CCR5 receptor; and (b) one compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate; wherein the relative mass ratio of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell.

2. A composition which comprises an admixture of three compounds, wherein: (a) one compound is an **antibody** or portion thereof which binds to a CCR5 receptor; (b) one compound retards attachment of **HIV**-1 to a **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell; and (c) one compound retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate; wherein the relative mass ratio of any two of the compounds in the admixture ranges from about 100:1 to about 1:100, the composition being effective to inhibit **HIV**-1 infection of the **CD4**+ cell.

3. The composition of claim 2, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a **CD4**-based protein.

4. The composition of claim 3, wherein the **CD4**-based protein is a **CD4**-immunoglobulin fusion protein.

5. The composition of claim 4, wherein the **CD4**-immunoglobulin fusion protein is **CD4**-IgG2, wherein the **CD4**-IgG2 comprises two heavy chains and two lights chains, wherein the heavy chains are encoded by an expression vector designated **CD4**-IgG2HC-pRcCMV (ATCC Accession No. 75193) and the light chains are encoded by an expression vector designated **CD4**-kLC-pRcCMV (ATCC Accession No. 75194).

6. The composition of claim 2, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a protein, the amino acid sequence of which comprises that of a protein found in **HIV**-1 as an envelope glycoprotein.

7. The composition of claim 6, wherein the protein binds to an epitope of **CD4** on the surface of the **CD4**+ cell.

8. The composition of claim 7, wherein the envelope glycoprotein is selected from the group consisting of **gp120**, **gp160**, and gp140.

9. The composition of claim 2, wherein the compound which retards the attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is an **antibody** or portion of an **antibody**.

10. The composition of claim 9, wherein the **antibody** is a monoclonal **antibody**.

11. The composition of claim 10, wherein the monoclonal **antibody** is a human, humanized or chimeric **antibody**.

12. The composition of claim 9, wherein the portion of the **antibody** is a Fab fragment of the **antibody**.

13. The composition of claim 9, wherein the portion of the **antibody** comprises the variable domain of the **antibody**.

14. The composition of claim 9, wherein the portion of the **antibody** comprises a CDR portion of the **antibody**.

15. The composition of claim 10, wherein the monoclonal **antibody** is an IgG, IgM, IgD, IgA, or IgE monoclonal **antibody**.

16. The composition of claim 10, wherein the monoclonal **antibody** binds to an **HIV**-1 envelope glycoprotein.

17. The composition of claim 16, wherein the **HIV**-1 envelope glycoprotein is selected from the group consisting of **gp120** and **gp160**.

18. The composition of claim 16, wherein **HIV**-1 envelope glycoprotein is **gp120** and the monoclonal **antibody** which binds to **gp120** is IgG1b12 or F105.

19. The composition of claim 9, wherein the **antibody** binds to an epitope of **CD4** on the surface of the **CD4**+ cell.

20. The composition of claim 2, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a peptide.

21. The composition of claim 2, wherein the compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell is a nonpeptidyl agent.

22. The composition of claim 1 or 2, wherein the compound which retards

gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is an **antibody**.

23. The composition of claim 22, wherein the **antibody** is a monoclonal **antibody**.

24. The composition of claim 1 or 2, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a peptide.

25. The composition of claim 1 or 2, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a fusion protein which comprises a peptide selected from the group consisting of T-20 (SEQ ID NO: 1), DP107 (SEQ ID NO: 2), N34 (SEQ ID NO: 3), C28 (SEQ ID NO: 4), N34(L6)C28 (SEQ ID NO: 5), and T-1249 (SEQ ID NO:6).

26. The composition of claim 24, wherein the peptide is selected from the group consisting of T-20 (SEQ ID NO: 1), DP107 (SEQ ID NO: 2), N34 (SEQ ID NO: 3), C28 (SEQ ID NO: 4), N34(L6)C28 (SEQ ID NO: 5), and T-1249 (SEQ ID NO:6).

27. The composition of claim 24, wherein the peptide is T-20 (SEQ ID NO: 1).

28. The composition of claim 1 or 2, wherein the compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate is a non-peptidyl agent.

29. The composition of claim 1 or 2, wherein the **antibody** which binds to a CCR5 receptor is selected from the group consisting of PA8 (ATCC Accession No. HB-12605), PA10 (ATCC Accession No.12607), PA11 (ATCC Accession No. HB-12608), PA12 (ATCC Accession No. HB-12609), and PA14 (ATCC Accession No. HB-12610).

30. The composition of claim 1 or 2, wherein the **antibody** is PA14 (ATCC Accession No. HB-12610).

31. The composition of claim 29, wherein the **antibody** is a monoclonal **antibody**.

32. The composition of claim 29, wherein the monoclonal **antibody** is a human, humanized or chimeric **antibody**.

33. The composition of claim 1 or 2, wherein the portion of the **antibody** is a Fab fragment of the **antibody**.

34. The composition of claim 1 or 2, wherein the portion of the **antibody** comprises the variable domain of the **antibody**.

35. The composition of claim 1 or 2, wherein the portion of the **antibody** comprises a CDR portion of the **antibody**.

36. The composition of claim 31, wherein the monoclonal **antibody** is an IgG, IgM, IgD, IgA, or IgE monoclonal **antibody**.

37. The composition of claim 1 or 2, wherein the relative mass ratio of each such compound in the admixture ranges from about 25:1 to about 1:1.

38. The composition of claim 37, wherein the mass ratio is about 25:1

39. The composition of claim 37, wherein the mass ratio is about 5:1.

40. The composition of claim 37, wherein the mass ratio is about 1:1.

41. The composition of claim 1 or 2, wherein the composition is admixed with a carrier.

42. The composition of claim 41, wherein the carrier is an aerosol, intravenous, oral or topical carrier.

43. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an amount of the composition of claim 1 or 2 effective to inhibit **HIV**-1 infection of the **CD4**+ cell so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

44. The method of claim 43, wherein the **CD4**+ cell is present in a subject and the contacting is effected by administering the composition to the subject.

45. The method of claim 43, wherein the effective amount of the composition comprises from about 0.000001 mg/kg body weight to about 100 mg/kg body weight of the subject.

46. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with (1) an amount of an **antibody** which binds to a CCR5 receptor and (2) an amount of a compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate, so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

47. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with (1) an amount of an **antibody** which binds to a CCR5 receptor, (2) an amount of a compound which retards attachment of **HIV**-1 to the **CD4**+ cell by retarding binding of **HIV**-1 **gp120** envelope glycoprotein to **CD4** on the surface of the **CD4**+ cell effective to inhibit **HIV**-1 infection of the **CD4**+ cell, and (3) an amount of a compound which retards gp41 from adopting a conformation capable of mediating fusion of **HIV**-1 to a **CD4**+ cell by binding noncovalently to an epitope on a gp41 fusion intermediate, so as to thereby inhibit **HIV**-1 infection of the **CD4**+ cell.

48. The method of claim 46 or 47, wherein the **CD4**+ cell is present in a subject and the contacting is effected by administering the compounds to the subject.

49. The method of claim 48, wherein the compounds are administered to the subject simultaneously.

50. The method of claim 48, wherein the compounds are administered to the subject at different times.

51. The method of claim 48, wherein the compounds are administered to the subject by different routes of administration.

L9 ANSWER 11 OF 16 USPATFULL on STN
2002:185564 Methods for characterizing the viral infectivity status of a host.
Hallowitz, Robert A., Newmarket, MD, UNITED STATES
Krowka, John, Frederick, MD, UNITED STATES
Matlock, Shawn, Frederick, MD, UNITED STATES
Bio-Tech Imaging, Inc., Frederick, MOLDOVA, REPUBLIC OF (U.S. corporation)
US 2002098476 A1 20020725
APPLICATION: US 2001-893604 A1 20010629 (9)
PRIORITY: US 2000-215075P 20000630 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods in accordance with the present invention involve novel measurements of the disease status of hosts infected with the **human immunodeficiency virus**. In particular, the present invention relates to a measurements of the numbers in a sample volume of (a) productively **HIV**-infected cells and (b) cells capable of being infected by **HIV**, e.g., cells expressing **CD4**, CCR5, and/or CXCR4. These two values can be represented as a single ratio, e.g., number of productively infected cells/number of cells capable of being infected by **HIV**, and can be utilized as an indicator of disease status, such as disease progression, viral replication, etc.

CLM What is claimed is:

1. A method of assessing the infectivity status of a host infected with **HIV**, comprising: measuring the number of cells in a sample which are expressing cell-surface **gp120** and the number of lymphocytes in said sample which are **CD4** positive, whereby the infectivity status of the host is assessed.

2. A method of claim 1, wherein the infectivity status is represented by the number of cells expressing cell-surface **gp120** per unit volume divided by the number of cells which are **CD4** positive per unit volume.

3. A method of claim 1, wherein the measuring is accomplished by flow cytometry.

4. A method of claim 1, wherein the measuring is accomplished by a fluorescence **resonance energy transfer** assay.

5. A method of claim 1, wherein the cells are peripheral blood mononuclear cells.

6. A method of claim 1, further comprising: combining an effective amount of an anti-**gp120 antibody** attached to a first detectable label and an effective amount of an anti-**CD4 antibody** attached to a second detectable label under conditions effective for said **antibodies** to bind **gp120** and **CD4** respectively.

7. A method of claim 6, wherein said measuring is accomplished by flow cytometry.

8. A method of claim 1, further comprising: combining an effective amount of an anti-**gp120 antibody** attached to a detectable label, an effective amount of an **antibody** specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said **gp120** to form a mixture, wherein said **antibody** specific-for said detectable label is attached to a magnetic particle; incubating said mixture under conditions effective for binding of said anti-**gp120 antibody** to **gp120** on said cells, and, for binding of said **antibody** specific-for said detectable label to said detectable label attached to said anti-**gp120 antibody**, to form a complex, wherein said anti-**gp120 antibody** is bound to said **gp120** displayed on a viral-infected cell; separating said complex by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field, and determining the presence of magnetically-separated cells by detecting said detectable label, whereby said magnetically separated cells are lymphocytes expressing cell-surface **gp120**.

9. A method of claim 1, wherein the **CD4** count of said host is less than 200/mm^3 of whole blood.

10. A method of claim 1, wherein the host has been treated with HAART.

11. A method of determining the infectivity status of a host infected with **HIV** virus who has tested negative in a virus co-culture assay, comprising: measuring the fraction of lymphocytes expressing cell-surface **gp120** and the fraction of lymphocytes which are **CD4** positive, whereby the infectivity status of the host is assessed.

12. A method of claim 11, wherein the measuring is accomplished by flow cytometry.

13. A method of claim 11, wherein the measuring is accomplished by a fluorescence **resonance energy transfer** assay.

14. A method of claim 11, wherein the cells are peripheral blood mononuclear cells.

15. A method of claim 11, further comprising: combining an effective amount of an anti-**gp120 antibody** attached to a first detectable label and an effective amount of an anti-**CD4 antibody** attached to a second detectable label under conditions effective for said **antibodies** to bind **gp120** and **CD4** respectively.

16. A method of claim 15, wherein said measuring is accomplished by flow cytometry.

L9 ANSWER 12 OF 16 USPATFULL on STN

2002:85121 Fluorescence **resonance energy transfer** screening assay for the identification of **HIV**-1 envelope glycoprotein-medicated cell.

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US 2002045161 A1 20020418
APPLICATION: US 2001-904356 A1 20010712 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides agents determined to be capable of inhibiting the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4**+cell and agents determined to be capable of inhibiting the fusion of a T cell-tropic isolate of **HIV**-1 to a **CD4**+cell. This invention also provides methods to identify such agents. This invention further provides methods of inhibiting fusion of a macrophage-tropic primary isolate of **HIV**-1 with a CD+ cell and methods of inhibiting fusion of a T cell-tropic isolate of **HIV**-1 with a **CD4**+cell susceptible to infection by a T cell-tropic isolate of **HIV**-1.

CLM What is claimed is:

1. A method for determining whether an agent is capable of inhibiting the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4**+ cell which comprises: (a) contacting (i) an appropriate **CD4**+ cell, which is labeled with a first dye, with (ii) a cell expressing the **HIV**-1 envelope glycoprotein of the macrophage-tropic primary isolate of **HIV**-1 on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the **CD4**+ cell to the cell expressing the **HIV**-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow **resonance energy transfer** between the dyes; (b) exposing the product of step (a) to conditions which would result in **resonance energy transfer** if fusion has occurred; and (c) determining whether there is a reduction of **resonance energy transfer**, when compared with the **resonance energy transfer** in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of **HIV**-1 to **CD4**+ cells.

2. The method of claim 1, wherein the **CD4**+ cell is a PM1 cell, a primary human T lymphocyte, or a primary human macrophage.

3. The method of claim 1, wherein the **HIV**-1 envelope glycoprotein+ cell is an **HIV**-1_{JR-FL} **gp120**/gp41 HeLa cell.

4. The method of claim 1 wherein the agent is not previously known.

5. An agent determined to be capable of inhibiting the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4**+ cell using the method of claim 1.

6. A therapeutic agent capable of inhibiting the fusion of an **HIV**-1 envelope glycoprotein+ cell with an appropriate **CD4**+ cell using the method of claim 1.

L9 ANSWER 13 OF 16 USPATFULL on STN
2001:218025 Compounds capable of inhibiting **HIV**-1 infection.
Litwin, Virginia M., Fayetteville, NY, United States
Allaway, Graham P., Cheshire, Great Britain
Maddon, Paul J., New York, NY, United States
Progenics Pharmaceuticals, Inc. (U.S. corporation)
US 2001046512 A1 20011129
APPLICATION: US 2001-891062 A1 20010625 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides an **antibody** capable of specifically inhibiting the fusion of an **HIV**-1 envelope glycoprotein cell with an appropriate **CD4**+ cell without cross reacting with the **HIV**-1 envelope glycoprotein or **CD4** and capable of inhibiting infection by one or more strains so **HIV**-1. This **antibody** is then used to identify a molecule which is important for **HIV** infection. Different uses of the **antibody** and the molecule are described.

CLM What is claimed is:

1. An **antibody** capable of specifically inhibiting the fusion of an **HIV**-1 envelope glycoprotein cell with an appropriate **CD4**+ cell without cross reacting with the **HIV**-1 envelope glycoprotein or **CD4** and capable of inhibiting infection by one or more strains of **HIV**-1.

2. A monoclonal **antibody** of claim i.

3. A hybridoma cell line producing the monoclonal **antibody** of claim 2.

4. A chimeric monoclonal **antibody** of claim 2.

5. A humanized monoclonal **antibody** of claim 4.

6. A human monoclonal **antibody** of claim 2.

7. A single chain **antibody** or an antigen binding **antibody** fragment of claim 2.

8. A monoclonal **antibody** capable of competitively inhibiting the binding of the monoclonal **antibody** of claim 2 to its target molecule.

9. The monoclonal **antibody** of claim 2, 4, 3, 6, 7, or 8 labelled with a detectable marker.

10. A monoclonal **antibody** of claim 9 wherein the detectable marker is a radioactive isotope, enzyme, dye or biotin.

11. A pharmaceutical composition comprising the complete or a portion of the monoclonal **antibody** of claim 2, 4, 5, 6, 7 or 8 and a pharmaceutically acceptable carrier.

12. A method of inhibiting **HIV**-1 infection in a subject comprising administering an effective amount of the pharmaceutical composition of claim 11 to the subject.

13. An isolated nucleic acid molecule encoding the complete or a portion of the light chain protein of the monoclonal **antibody** of claim 2, 4, 5, 6 or 8.

14. An isolated nucleic acid molecule encoding the complete of a portion of the heavy chain protein of the monoclonal **antibody** of claim 2, 4, 5, 6 or 8.

15. An isolated nucleic acid molecule encoding the single chain **antibody** of claim 7.

16. A vector comprising the nucleic acid molecule of claim 13, 14 or 15 operably linked to a promoter of RNA transcription.

17. A vector comprising the nucleic acid molecules of claims 13 and 14 each operably linked to a promoter of RNA transcription.

18. A host vector system comprising one or more vectors of claim 16 or 17 in a suitable host cell.

19. A host vector system of claim 18, wherein the suitable host cell is selected from a group consisting of a bacterial cell, an insect cell, a yeast cell or a mammalian cell.

20. The molecule specifically recognized by nhe monoclonal **antibody** of claim 1.

21. A glycoloipd molecule of claim 20.

22. A polypeptide molecule of claim 20.

23. An isolated nucleic acid molecule encoding the complete or a portion of the polypeptide of claim 22.

24. A multichain polypeptide molecule comprising the polypeptide of claim 22.

25. A soluble protein comprising a portion of the polypep-ide of claim 22 or 24.

26. A pharmaceutical composition comprising an effective amount of the soluble protein of claim 25 to inhibit **HIV**-1 infection and a pharmaceutically acceptable carrier.

27. A method of inhibiting **HIV**-1 infection in a subject comprising administering an effective amount of the pharmaceutical composition of claim 26 to the subject.

28. An isolated nucleic acid molecule encoding the complete or a portion of a polypeptide of the multichain polypeptide molecule of claim 24.

29. A vector comprising the nucleic acid molecule of claim 23 or 28 operably linked to a promoter of RNA transcription.

30. A host vector system comprising the vector of claim 29 in a suitable host cell.

31. A host vector system of claim 30, wherein the suitable host cell is a selected from a group consisting of a bacterial cell, an insect cell, a yeast cell or a mammalian cell.

32. A method for identifying inhibitors of **HIV**-1 infection comprising steps of: (a) contacting an effective amount of a compound with a system which contains **HIV**-1 **gp120**, **HIV**-1 gp41 or a fragment thereof with the molecule of claim 20 under conditions permitting formation of a complex between **HIV**-1 **gp120**, **HIV**-1 gp41 or a fragment thereof and the molecule, so as to inhibit such formation; and (b) determining the amount of complex formed; and (c) comparing the amount determined in step (b) with the control which is without the

addition of the compound, a decrease in the complex formation indicating that the compound is capable of inhibiting **HIV**-1 infection.

33. A method of claim 32, wherein the compound is not previously known.

34. The compound identified by claim 33.

35. A pharmaceutical composition comprising the compound identified by the method of claim 32 and a pharmaceutically acceptable carrier.

36. A method of inhibiting **HIV**-1 infection in a subject comprising administering an effective amount of the pharmaceutical composition of claim 35 to the subject.

37. A kit for identifying inhibitors of **HIV**-1 infection which comprises, in separate compartments: (a) purified **HIV**-1 **gp120**, **HIV**-1 gp41 or a fragment thereof; and (b) the molecule of claim 20.

38. A transgenio nonhuman animal which comprises an isolated DNA molecule encoding the molecule of claim 22 or 24.

39. The transgenic nonhuman animal of claim 38 further comprising an isolated DNA molecule encoding the full-length or portion of the **CD4** molecule sufficient for binding the **HIV**-1 envelope glycoprotein.

L9 ANSWER 14 OF 16 USPATFULL on STN

2001:112032 Fluorescence **resonance energy transfer** screening assay for the identification of compounds that are capable of abrogating macrophage-tropic **HIV**-1 cell fusion.

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US 6261763 B1 20010717
WO 9641020 19961219
APPLICATION: US 1998-973601 19980316 (8)
WO 1996-US9894 19960607 19980316 PCT 371 date 19980316 PCT 102(e) date
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Previous studies of **human immunodeficiency virus** type 1 (**HIV**-1) envelope glycoprotein-mediated membrane fusion have focused on laboratory-adapted T-lymphotropic strains of the virus. The goal of this application was to develop a novel screening assay to characterize membrane fusion mediated by a primary **HIV**-1 isolate in comparison with a laboratory-adapted strain. To this end, a novel fusion assay was developed on the basis of the principle of **resonance energy transfer**, using HeLa cells stably transfected with **gp120**/gp41 from the T-lymphotropic isolate **HIV**-1_{LAI} or the macrophage-tropic primary isolate **HIV**-1_{JR-FL}. These cells fused with **CD4**+ target cell lines with a tropism mirroring that of infection by the two viruses. Of particular note, HeLa cells expressing **HIV**-1_{JR-FL} **gp120**/gp41 fused only with PM1 cells, a clonal derivative of HUT 78, and not with other T-cell or macrophage cell lines. These results demonstrate that the envelope glycoproteins of these strains play a major role in mediating viral tropism. Despite significant differences exhibited by **HIV**-1_{JR-FL} and **HIV**-1_{LAI} in terms of tropism and sensitivity to neutralization by **CD4**-based proteins, the present study found that membrane fusion mediated by the envelope glycoproteins of these viruses had remarkably similar properties. In particular, the degree and kinetics of membrane fusion were similar, fusion occurred at neutral pH and was dependent on the presence of divalent cations. The claimed invention will facilitate the screening and identification of novel agents that are capable of inhibiting these interactions.

CLM What is claimed is:

1. A method for determining whether an agent is capable of specifically inhibiting (A) the fusion of a macrophage-tropic primary isolate of **HIV**-1 to a **CD4**+ cell susceptible to infection by a macrophage-tropic **HIV**-1 or (B) the fusion of a T cell-tropic isolate of **HIV**-1 to a cell susceptible to infection by a T cell tropic **HIV**-1, but not both, which comprises: (a) contacting (i) a first appropriate **CD4**+ cell, which is labeled with a first dye, with (ii) a cell expressing the **HIV**-1 envelope glycoprotein of the macrophage-tropic primary isolate of **HIV**-1 on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions which would normally permit the fusion of the **CD4**+ cell to the cell expressing the **HIV**-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow **resonance energy transfer** between the dyes; (b) exposing the result of step (a) to conditions which would result in **resonance energy transfer** if fusion has occurred; and (c) determining whether there is a reduction of **resonance energy transfer**, when compared with the **resonance energy transfer** in the absence of the agent; (d) contacting (i) a second appropriate **CD4**+ cell, which is labeled with a first dye, with (ii) a cell expressing the **HIV**-1 envelope glycoprotein of a T cell-tropic isolate of **HIV**-1 on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions which would normally permit the fusion of the **CD4**+ cell to the cell expressing the **HIV**-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow **resonance energy transfer** between the dyes; (e) exposing the result of step (d) to conditions which would result in **resonance energy transfer** if fusion has occurred; and (f) determining whether there is a reduction of **resonance energy transfer**, when compared with the **resonance energy transfer** in the absence of the agent, wherein a decrease in transfer in step (c) but not step (f) indicates that the agent is capable of specifically inhibiting fusion of the macrophage-tropic primary isolate of **HIV**-1 to **CD4**+ cells and a decrease in transfer in step (f) but not step (c) indicates that the agent is capable of specifically inhibiting the fusion of a T cell-tropic isolate of **HIV**-1 to the **CD4**+ cells.

2. The method of claim 1, wherein the first appropriate **CD4**+ cell is a PM1 cell, a primary human T lymphocyte, or a primary human macrophage.

3. The method of claim 1, wherein the second appropriate **CD4**+ cell is a HeLa-**CD4** cell, a primary human T lymphocyte, a human T cell line, PM1 cell, or a C8166 cell.

4. The method of claim 1, wherein in step (a) the cell expressing the **HIV**-1 envelope glycoprotein of the macrophage-tropic primary isolate is an **HIV**-1_{JR-FL} **gp120**/gp41 HeLa cell.

5. The method of claim 1, wherein in step (d) the cell expressing the **HIV**-1 envelope glycoprotein of the T-cell-tropic of **HIV**-1 is an **HIV**-1_{LAI} **gp120**/gp41 HeLa cell.

6. The method of claim 1 wherein the agent is not previously known.

7. The method of claim 1, wherein the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

8. The method of claim 7, wherein the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

9. The method of claim 1, wherein the first dye is a fluorescein

moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

L9 ANSWER 15 OF 16 USPATFULL on STN
2000:109525 Method for preventing **HIV**-1 infection of **CD4+** cells.
Allaway, Graham P., Mohegan Lake, NY, United States
Litwin, Virginia M., Fayetteville, NY, United States
Maddon, Paul J., Elmsford, NY, United States
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US 6107019 20000822
APPLICATION: US 1997-876078 19970613 (8)
PRIORITY: US 1996-19715P 19960614 (60)
US 1996-14532P 19960402 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods for inhibiting fusion of **HIV**-1 to **CD4+** cells which comprise contacting **CD4+** cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4+** cells is inhibited. This invention also provides methods for inhibiting **HIV**-1 infection of **CD4+** cells which comprise contacting **CD4+** cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4+** cells is inhibited, thereby inhibiting the **HIV**-1 infection. This invention provides non-chemokine agents capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4+** cells. This invention also provides pharmaceutical compositions comprising an amount of the non-chemokine agent capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4+** cells effective to prevent fusion of **HIV**-1 to **CD4+** cells and a pharmaceutically acceptable carrier.

CLM What is claimed is:
1. An in vitro method for determining whether an agent is capable of inhibiting **HIV**-1 infection of a **CD4+** cell susceptible to **HIV**-1 infection comprising the steps of: (a) fixing a chemokine receptor on a solid matrix wherein the chemokine receptor is a co-receptor for **HIV**-1 infection; (b) contacting the fixed chemokine receptor with the agent under conditions permitting binding of the agent to the chemokine receptor; (c) removing any unbound agent; (d) contacting the resulting fixed chemokine receptor to which the agent is bound with a predetermined amount of **gp120/CD4+** complex under conditions permitting binding of **gp120/CD4** + complex to the fixed chemokine receptor in the absence of the agent; (e) removing any unbound **gp120/CD4+** complex; (f) measuring the amount of **gp120/CD4+** complex bound to the fixed chemokine receptor; and (g) comparing the amount measured in step (f) with the amount measured in the absence of the agent, a decrease in the amount bound in the presence of the agent indicating that the agent is capable of inhibiting **HIV**-1 infection.

2. An in vitro method for determining whether an agent is capable of inhibuting **HIV**-1 infection of a **CD4+** cell susceptible to **HIV**-1 infection comprising the steps: (a) fixing a chernokine receptor on a solid matrix wherein the chemokine receptor is a co-receptor for **HIV**-1 infection; (b) contacting the fixed chemokine receptor with the agent and a predetermined amount of **gp120/CD4+** complex under conditions permitting binding of the **gp120/CD4+** complex to the fixed chemokine receptor in the absence of the agent; (c) removing any unbound agent or unbound **gp120/CD4+** complex or both; (d) measuring the amount of **gp120/CD4+** complex bound to the fixed chemokine receptor; and (e) comparing the amount measured in step (d) with the amount measured in the absence of the agent, a decrease in the amount bound in the presence of the agent indicating that the agent is

capable of inhibiting **HIV**-1 infection.

3. An in vitro method for determining whether an agent is capable of inhibiting **HIV**-1 infection of a **CD4**+ cell susceptible to **HIV**-1 infection comprising steps of: (a) fixing a **gp120/CD4**+ complex on a solid matrix; (b) contacting the fixed **gp120/CD4**+ complex with the agent under conditions permitting the binding of the agent to the **gp120/CD4**+ complex; (c) removing any unbound agent; (d) contacting the resulting fixed **gp120/CD4**+ complex to which the agent is bcund with a predetermined amount of chemokine receptor, wherein the chemokine receptor is a co-receptor for **HIV**-1 infection, under conditions permitting binding of the chemokine receptor to the fixed the **gp120/CD4**+ complex in the absence of the agent; (e) removing any unbound chemokine receptor; (f) measuring the amount of chemokine receptor bound to the fixed **gp120/CD4**+ ; and (g) comparing the amount measured in step (f) with the amount measured in the absence of the agent, a decrease in the amount bound in the presence of the agent indicating that the agent is capable of inhibiting **HIV**-1 infection.

4. An in vitro method for determining whether an agent is capable of inhibiting **HIV**-1 infection of a **CD4**+ cell susceptible to **HIV**-1 infection comprising steps of: (a) fixing a **gp120/CD4**+ complex on a solid matrix: (b) contacting the fixed **gp120/CD4**+ complex with the agent and a predetermined amount of chemokine receptor, wherein the chemokine receptor is a co-receptor for **HIV**-1 infection, under co)nditions permitting binding of the chemokine receptor to the fixed **gp120/CD4**+ complex in the absence of the agent; (c) removing any unbound agent or any unbound chemokine receptor or both: (d) measuring the amount of chemorkine receptor bound to the fixed **gp120/CD4**+ ; and (e) comparing the amount measured in step (d) with the amount measured in the absence of the agent, a decrease in the amount bound in the presence of the agent indicating that the agent is capable of inhibiting **HIV**-1 infection.

5. The method of claim 1, 2, 3, or 4 wherein the **CD4**+ is a soluble **CD4**+.

6. The method of claim 1, 2, 3, or 4 wherein the chemokine receptor is expressed on a cell.

7. The method of claim 6 wherein the cell is a L1.2 cell.

8. The method of claim 1 or 2, wherein the **gp120**, **CD4**+ or both are labeled with a detectable marker.

9. The method of claim 3 or 4 wherein the chemokine receptor is labeled with a detectable marker.

10. The method of claim 1 or 2, wherein the **gp120**, **CD4**+ or both are labeled with biotin.

11. The method of claim 2 or 4 wherein the chemokine receptor is labeled with biotin.

12. The method of any one of claims 1, 2, 3, or 4, wherein the chemockine receptor is CCR5.

=> d his

(FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004)

FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
E HALLOWITZ R A/IN

```
L1             7 S E4 OR E5
                 E KROWKA JOHN/IN
L2             1 S E3
                 E MATLOCK SHAWN/IN
L3             2 S E3 OR E4
L4         33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5          3422 S L4 AND (GP120 OR GP160)
L6          2490 S L5 AND (CD4?)
L7           182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8           182 S L7 AND ANTIBOD?
L9            16 S L8 AND (GP120/CLM OR GP160/CLM)

=> s l7 and ay<2000
       2994197 AY<2000
L10          20 L7 AND AY<2000

=> s l10 not l9
L11          15 L10 NOT L9

=> d l11,ti,1-15

L11  ANSWER 1 OF 15  USPATFULL on STN
TI     Methods for the inhibition of epstein-barr virus transmission employing
       anti-viral peptides capable of abrogating viral fusion and transmission

L11  ANSWER 2 OF 15  USPATFULL on STN
TI     Methods and compositions for making dendritic cells from expanded
       populations of monocytes and for activating T cells

L11  ANSWER 3 OF 15  USPATFULL on STN
TI     Methods for inhibition of membrane fusion-associated events, including
       respiratory syncytial virus transmission

L11  ANSWER 4 OF 15  USPATFULL on STN
TI     MODIFIED HIV ENV POLYPEPTIDES

L11  ANSWER 5 OF 15  USPATFULL on STN
TI     Method for preventing HIV-1 infection of CD4+ cells

L11  ANSWER 6 OF 15  USPATFULL on STN
TI     Immunogenic peptides of prostate specific antigen

L11  ANSWER 7 OF 15  USPATFULL on STN
TI     Protein fragment complementation assays for the detection of biological
       or drug interactions

L11  ANSWER 8 OF 15  USPATFULL on STN
TI     Protein fragment complementation assays for the detection of biological
       or drug interactions

L11  ANSWER 9 OF 15  USPATFULL on STN
TI     Human respiratory syncytial virus peptides with antifusogenic and
       antiviral activities

L11  ANSWER 10 OF 15  USPATFULL on STN
TI     Chimeric Gag pseudovirions

L11  ANSWER 11 OF 15  USPATFULL on STN
TI     Method and apparatus for detecting cancer, influenza, or HIV based on
       α-N-acetyl-galactosaminidase detection

L11  ANSWER 12 OF 15  USPATFULL on STN
TI     Diagnostic and prognostic ELISA assays of serum α-N-
       acetylgalactosaminidase for AIDS

L11  ANSWER 13 OF 15  USPATFULL on STN
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TI     Spiro-substituted azacycles as modulators of chemokine receptor activity

L11  ANSWER 14 OF 15  USPATFULL on STN
TI     Immunogenic peptides of prostate specific antigen

L11  ANSWER 15 OF 15  USPATFULL on STN
TI     Diagnostic and prognostic ELISA assays of serum α-N-
       acetylgalactosaminidase for influenza


=> d his

     (FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
                E HALLOWITZ R A/IN
L1            7 S E4 OR E5
                E KROWKA JOHN/IN
L2            1 S E3
                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
L7          182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8          182 S L7 AND ANTIBOD?
L9           16 S L8 AND (GP120/CLM OR GP160/CLM)
L10          20 S L7 AND AY<2000
L11          15 S L10 NOT L9

=> d l11,cbib,ab,clm,4,5

L11  ANSWER 4 OF 15  USPATFULL on STN
2002:265807 MODIFIED HIV ENV POLYPEPTIDES.
    BARNETT, SUSAN, EMERVILLE, CA, UNITED STATES
    HARTOG, KARIN, EMERYVILLE, CA, UNITED STATES
    MARTIN, ERIC, EMERVILLE, CA, UNITED STATES
    US 2002146683 A1 20021010
    APPLICATION: US 1999-476242  A1  19991230 (9)                    <--
    PRIORITY: US 1998-114495P  19981231 (60)
    US 1999-156670P  19990929 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Polynucleotide encoding modified HIV Env polypeptides are disclosed.
       The Env polypeptides are modified so as to expose at least part of the
       CD4 binding region. Methods of diagnosis, treatment and prevention
       using the polynucleotides and polypeptides are also provided.
CLM    What is claimed is:
       1. A polynucleotide encoding a modified HIV Env polypeptide wherein
       the polypeptide has at least one amino acid deleted or replaced in the
       region corresponding to residues 420 to 436 relative to HXB-2 (SEQ ID
       NO:1).

       2. The polynucleotide of claim 1, wherein the region corresponding to
       residues 124-198 relative to HXB-2 is deleted and at least one amino
       acid is deleted or replaced in the regions corresponding to the residues
       119 to 123 and 199 to 210 relative to HXB-2 (SEQ ID NO: 1).

       3. The polynucleotide of claim 1, wherein at least one amino acid in the
       region corresponding to residues 427 through 429 relative to HXB-2 (SEQ
       ID NO: 1) is deleted or replaced.

       4. The polynucleotide of claim 2, wherein at least one amino acid of the
       in the region corresponding to residues 427 through 429 relative to
       HXB-2 (SEQ ID NO: 1) is deleted or replaced.
```

5. The polynucleotide of claim 1, wherein the amino acid sequence of the modified **HIV** Env polypeptide is based on strain SF162.

6. An immunogenic modified **HIV** Env polypeptide having at least one amino acid deleted or replaced in the region corresponding to residues 420 through 436, relative to HXB-2 (SEQ ID NO:1).

7. The polypeptide of claim 6, wherein one amino acid is deleted in the region corresponding to residues 420 through 436, relative to HXB-2 (SEQ ID NO: 1).

8. The polypeptide of claim 6, wherein more than one amino acid is deleted in the region corresponding to residues 420 through 436, relative to HXB-2 (SEQ ID NO:1).

9. The polypeptide of claim 6, wherein at least one amino acid is replaced in the region corresponding to residues 420 through 436, relative to HXB-2 (SEQ ID NO: 1).

10. The polypeptide of claim 6, wherein at least one amino acid residue between about amino acid residue 427 and amino acid residue 429 relative to HXB-2 (SEQ ID NO: 1) is deleted or replaced.

11. The polypeptide of claim 6, wherein the V1 and V2 regions of the polypeptide are truncated.

12. The polypeptide of claim 10, wherein the V1 and V2 regions of the polypeptide are truncated.

13. The polypeptide of claim 6, wherein the amino acid sequence of the modified **HIV** Env polypeptide is based on strain SF162.

14. A construct comprising the nucleotide sequence depicted in FIG. 6 (SEQ ID NO:3).

15. A construct comprising the nucleotide sequence depicted in FIG. 7 (SEQ ID NO:4).

16. A construct comprising the nucleotide sequence depicted in FIG. 8 (SEQ ID NO:5).

17. A construct comprising the nucleotide sequence depicted in FIG. 9 (SEQ ID NO:6).

18. A construct comprising the nucleotide sequence depicted in FIG. 10 (SEQ ID NO:7).

19. A construct comprising the nucleotide sequence depicted in FIG. 11 (SEQ ID NO:8).

20. A construct comprising the nucleotide sequence depicted in FIG. 12 (SEQ ID NO:9).

21. A construct comprising the nucleotide sequence depicted in FIG. 13 (SEQ ID NO:10).

22. A construct comprising the nucleotide sequence depicted in FIG. 14 (SEQ ID NO: 11).

23. A construct comprising the nucleotide sequence depicted in FIG. 15 (SEQ ID NO: 12).

24. A construct comprising the nucleotide sequence depicted in FIG. 16 (SEQ ID NO:13).

25. A construct comprising the nucleotide sequence depicted in FIG. 17

(SEQ ID NO:14).

26. A construct comprising the nucleotide sequence depicted in FIG. 18 (SEQ ID NO:15).

27. A construct comprising the nucleotide sequence depicted in FIG. 19 (SEQ ID NO: 16).

28. A construct comprising the nucleotide sequence depicted in FIG. 20 (SEQ ID NO:17).

29. A construct comprising the nucleotide sequence depicted in FIG. 21 (SEQ ID NO:18).

30. A construct comprising the nucleotide sequence depicted in FIG. 22 (SEQ ID NO:19).

31. A construct comprising the nucleotide sequence depicted in FIG. 23 (SEQ ID NO:20).

32. A construct comprising the nucleotide sequence depicted in FIG. 24 (SEQ ID NO:21).

33. A construct comprising the nucleotide sequence depicted in FIG. 25 (SEQ ID NO:22).

34. A construct comprising the nucleotide sequence depicted in FIG. 26 (SEQ ID NO:23).

35. A construct comprising the nucleotide sequence depicted in FIG. 27 (SEQ ID NO:24).

36. A construct comprising the nucleotide sequence depicted in FIG. 28 (SEQ ID NO:25).

37. A construct comprising the nucleotide sequence depicted in FIG. 29 (SEQ ID NO:26).

38. A vaccine composition comprising a polynucleotide encoding a modified Env polypeptide according to claim 1.

39. A vaccine composition comprising a polynucleotide encoding a modified Env polypeptide according to claim 2.

40. A vaccine composition comprising a polynucleotide encoding a modified Env polypeptide according to claim 3.

41. A vaccine composition comprising a polynucleotide encoding a modified Env polypeptide according to claim 4.

42. A vaccine composition comprising a modified Env polypeptide according to claim 6 and an adjuvant.

43. A vaccine composition comprising a modified Env polypeptide according to claim 10 and an adjuvant.

44. A method of inducing an immune response in subject comprising, administering a polynucleotide according to claim 1 in an amount sufficient to induce an immune response in the subject.

45. The method of claim 44 further comprising administering an adjuvant to the subject.

46. A method of inducing an immune response in a subject comprising administering a composition comprising a modified Env polypeptide according to claim 6 and an adjuvant, wherein the composition is

administered in an amount sufficient to induce an immune response in the subject.

47. A method of inducing an immune response in a subject comprising (a) administering a first composition comprising a polynucleotide according to claim 1 in a priming step and (b) administering a second composition comprising a modified Env polypeptide according to claim 6, as a booster, in an amount sufficient to induce an immune response in the subject.

48. The method of claim 47 wherein the first composition or second composition further comprise an adjuvant.

49. The method of claim 48 wherein the first and second compositions further comprise an adjuvant.

L11 ANSWER 5 OF 15 USPATFULL on STN
2002:24365 Method for preventing **HIV**-1 infection of **CD4**+ cells.
Allaway, Graham P., Mohegan Lake, NY, United States
Litwin, Virginia M., Fayetteville, NY, United States
Maddon, Paul J., Elmsford, NY, United States
Olson, William C., Ossining, NY, United States
Progenics Pharmaceuticals, Inc., Tarrytown, NY, United States (U.S. corporation)
US 6344545 B1 20020205
APPLICATION: US 1997-831823 19970402 (8) <--
PRIORITY: US 1996-19715P 19960614 (60)
US 1996-14532P 19960402 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods for inhibiting fusion of **HIV**-1 to **CD4**+ cells which comprise contacting CD^4+ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited. This invention also provides methods for inhibiting **HIV**-1 infection of **CD4**+ cells which comprise contacting **CD4**+ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of **HIV**-1 to the **CD4**+ cells is inhibited, thereby inhibiting the **HIV**-1 infection. This invention provides non-chemokine agents capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells. This invention also provides pharmaceutical compositions comprising an amount of the non-chemokine agent capable of binding to the chemokine receptor and inhibiting fusion of **HIV**-1 to **CD4**+ cells effective to prevent fusion of **HIV**-1 to **CD4**+ cells and a pharmaceutically acceptable carrier.

CLM What is claimed is:
1. A method of inhibiting **HIV**-1 infection of a **CD4**+ cell which comprises contacting the **CD4**+ cell with an antibody or portion of an antibody capable of binding to a chemokine receptor on the surface of the **CD4**+ cell in an amount and under conditions such that fusion of **HIV**-1 or an **HIV**-1 infected cell to the **CD4**+ cell is inhibited, thereby inhibiting **HIV**-1 infection of the **CD4**+ cell.

2. The method of claim 1, wherein the chemokine receptor is a CCR5 chemokine receptor.

3. The method of claim 1, wherein the **CD4**+ cell is a PM-1 cell.

4. The method of claim 1, wherein the **CD4**+ cell is a primary **CD4**+ T-cell.

5. The method of claim 1, wherein the **CD4**+ cell is a PMBC cell.

6. The method of claim 1, wherein the antibody is a monoclonal antibody.

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=> d his

     (FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
                E HALLOWITZ R A/IN
L1            7 S E4 OR E5
                E KROWKA JOHN/IN
L2            1 S E3
                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
L7          182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8          182 S L7 AND ANTIBOD?
L9           16 S L8 AND (GP120/CLM OR GP160/CLM)
L10          20 S L7 AND AY<2000
L11          15 S L10 NOT L9

=> s s l6 and antibod?
MISSING OPERATOR S L6
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l6 and antibod?
        103369 ANTIBOD?
L12       2375 L6 AND ANTIBOD?

=> s l12 and (antibod?/clm)
         31312 ANTIBOD?/CLM
L13       1113 L12 AND (ANTIBOD?/CLM)

=> s l13 and (green fluorescent protein or GFP or renilla luciferase or Rluc)
        225080 GREEN
        119830 FLUORESCENT
        174002 PROTEIN
          7776 GREEN FLUORESCENT PROTEIN
                 (GREEN(W)FLUORESCENT(W)PROTEIN)
          7294 GFP
           768 RENILLA
         15703 LUCIFERASE
           420 RENILLA LUCIFERASE
                 (RENILLA(W)LUCIFERASE)
            29 RLUC
L14        211 L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERASE
               OR RLUC)

=> s l14 and ay<2001
       3177390 AY<2001
L15         19 L14 AND AY<2001

=> d l15,ti,1-19

L15  ANSWER 1 OF 19  USPATFULL on STN
TI    Determining viral load in double negative T cells

L15  ANSWER 2 OF 19  USPATFULL on STN
TI    Viral vectors with modified tropism

L15  ANSWER 3 OF 19  USPATFULL on STN
TI    Anti-inflammatory therapy for inflammatory mediated infection
```

L15 ANSWER 4 OF 19 USPATFULL on STN
TI Polynucleotides encoding flavivirus and alphavirus multivalent antigenic polypeptides

L15 ANSWER 5 OF 19 USPATFULL on STN
TI Flavivirus and alphavirus recombinant antigen libraries

L15 ANSWER 6 OF 19 USPATFULL on STN
TI Methods of identifying g-couple receptors associated with macrophage-thophic **hiv**, and diagnostic and therapeutic uses thereof

L15 ANSWER 7 OF 19 USPATFULL on STN
TI Targeting recombinant virus with a bispecific fusion protein ligand in coupling with an **antibody** to cells for gene therapy

L15 ANSWER 8 OF 19 USPATFULL on STN
TI Compositions and methods for identifying antigens which elicit an immune response

L15 ANSWER 9 OF 19 USPATFULL on STN
TI ANTIGEN LIBRARY IMMUNIZATION

L15 ANSWER 10 OF 19 USPATFULL on STN
TI Recombinant Rhabdovirus containing a heterologous fusion protein

L15 ANSWER 11 OF 19 USPATFULL on STN
TI USE OF NEURONAL APOPTOSIS INHIBITOR PROTEIN (NAIP)

L15 ANSWER 12 OF 19 USPATFULL on STN
TI CD20-specific redirected T cells and their use in cellular immunotherapy of CD20+ malignancies

L15 ANSWER 13 OF 19 USPATFULL on STN
TI Hydrogel compositions for controlled delivery of virus vectors and methods of use thereof

L15 ANSWER 14 OF 19 USPATFULL on STN
TI METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD

L15 ANSWER 15 OF 19 USPATFULL on STN
TI REAGENT SYSTEM AND KIT FOR DETECTING **HIV** INFECTED CELLS

L15 ANSWER 16 OF 19 USPATFULL on STN
TI Methods of identifying g-coupled receptors associated with macrophage-trophic **HIV**, and diagnostic and therapeutic uses thereof

L15 ANSWER 17 OF 19 USPATFULL on STN
TI GENETIC VACCINE VECTOR ENGINEERING

L15 ANSWER 18 OF 19 USPATFULL on STN
TI XAF genes and polypeptides: methods and reagents for modulating apoptosis

L15 ANSWER 19 OF 19 USPATFULL on STN
TI Composition and method for detecting **HIV** with baculovirus derived vesicles

=> d l15,cbib,ab,clm,1,14,15

L15 ANSWER 1 OF 19 USPATFULL on STN
2003:326920 Determining viral load in double negative T cells.
Posnett, David N., New York, NY, United States
Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)
US 6664042 B1 20031216

WO 2000043551 20000727
APPLICATION: US 2001-890010 20011123 (9) <--
WO 2000-US1959 20000126 <--
PRIORITY: US 1999-117447P 19990126 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a method for determining viral load in a patient infected with **human immunodeficiency virus**, which is useful in patients where viral loads are not detectable in plasma. The levels of **human immunodeficiency virus** are measured in **CD4**$^{-CD8-}$ double negative cells. Furthermore, the invention also provides a kit for determining viral load in a patient infected with **human immunodeficiency virus**.

CLM What is claimed is:

1. A method for determining viral load in a patient infected with **human immunodeficiency virus**, comprising: measuring the levels of **human immunodeficiency virus** in **CD4**$^-$ CD8$^-$ T cells.

2. The method according to claim 1, further comprising: isolating T cells; isolating **CD4**$^-$ CD8$^-$ cells; and measuring the levels of **human immunodeficiency virus** in the isolated **CD4**$^-$ CD8$^-$ T cells.

3. The method according to claim 2, wherein the isolating the T cells comprises: isolating cells having a T cell specific marker.

4. The method according to claim 3, wherein the T cell specific marker is selected from the group consisting of CD2+, CD3+, and T cell receptor $\alpha\beta$.

5. The method according to claim 4, wherein the T cell specific marker is CD3+.

6. The method according to claim 2, wherein the isolating the T cells is carried out by removing CD3+ T cells using magnetic beads coated with **antibodies** specific for T cells.

7. The method according to claim 2, wherein the isolating the T cells is carried out by separating T cells using flourescence activated cell sorting.

8. The method according to claim 2, wherein the isolating the T cells is carried out by removing T cells using a panning procedure.

9. The method according to claim 2, wherein the isolating the **CD4**$^-$ and CD8$^-$ T cells is carried out by removing **CD4**+ and CD8+ T cells.

10. The method according to claim 9, wherein the isolating the **CD4**$^-$ and CD8$^-$ T cells is carried using magnetic beads coated with **antibodies** specific for **CD4**+ and CD8+ T cells.

11. The method according to claim 9, wherein the isolating the **CD4**$^-$ and CD8$^-$ T cells is carried out using flourescence activated cell sorting.

12. The method according to claim 9, wherein the isolating the **CD4**$^-$ and CD8$^-$ T cells is carried out using a panning procedure.

13. The method according to claim 2, wherein the measuring the levels of **human immunodeficiency virus** is carried out by measuring levels of **human immunodeficiency virus** DNA, **human immunodeficiency virus** RNA, or **human immunodeficiency virus** proteins.

14. The method according to claim 13, wherein the measuring the levels of **human immunodeficiency virus** is carried out by measuring levels of **human immunodeficiency virus** DNA.

15. The method according to claim 14, wherein the measuring levels of **human immunodeficiency virus** DNA is carried out by sequence specific hybridization.

16. The method according to claim 15, wherein sequence specific hybridization utilizes probes specific to a portion of the gag or pol genes.

17. The method according to claim 14, wherein the measuring levels of **human immunodeficiency virus** DNA further comprises, amplification of the **human immunodeficiency virus** DNA by polymerase chain reaction.

18. The method according to claim 13, wherein the measuring the levels of **human immunodeficiency virus** is carried out by measuring levels of **human immunodeficiency virus** RNA.

19. The method according to claim 18, wherein the measuring levels of **human immunodeficiency virus** RNA is carried out by sequence specific hybridization.

20. The method according to claim 19, wherein sequence specific hybridization utilizes probes specific to a portion of the gag or pol genes.

21. The method according to claim 18, wherein the measuring levels of **human immunodeficiency virus** RNA further comprises, amplification of the **human immunodeficiency virus** RNA by polymerase chain reaction.

22. The method according to claim 18, wherein the **human immunodeficiency virus** specific transcripts are unspliced viral mRNA transcripts.

23. The method according to claim 18, wherein the **human immunodeficiency virus** specific transcripts are multispliced viral mRNA transcripts.

24. The method according to claim 13, wherein the measuring the levels of **human immunodeficiency virus** is carried out by measuring levels of **human immunodeficiency virus** protein.

25. The method according to claim 24, wherein the measuring levels of **human immunodeficiency virus** protein comprises: contacting a sample from the patient with a binding protein which specifically binds to a **human immunodeficiency virus** protein; and determining the amount of binding protein which binds to the **human immunodeficiency virus** protein.

26. The method according to claim 25, wherein the binding protein is an **antibody**.

27. The method according to claim 26, wherein the **antibody** binds to Nef, Env, or Vpu.

28. The method according to claim 25, wherein the binding protein is a T cell receptor.

29. The method according to claim 28, wherein the T cell receptor is **CD4**.

30. The method according to claim 1, wherein the patient is being treated with highly active retroviral therapy.

31. The method according to claim 30, wherein the patient has no detectable plasma viral load.

L15 ANSWER 14 OF 19 USPATFULL on STN
2001:199904 METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD.
HALLOWITZ, ROBERT, GAITHERSBURG, MD, United States
SALAS, VIRGINIA, ALBUQUERQUE, NM, United States
US 2001039007 A1 20011108
APPLICATION: US 1999-296534 A1 19990422 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a new **HIV** status of a patient called "latent viral load." To measure the "latent viral load," in accordance with a preferred embodiment of the present invention, a population of sample cells is obtained from a desired source, such as an infected patient. The sample cell population is depleted of overtly infected cells and cells harboring active virus, to produce a subset of "resting cells" comprising uninfected and latently-infected cells. This subset is treated with an agent and/or condition that activates the latent virus in the host cell genome and results in a productive infection. The thus-produced infection reflects the "latent viral load" of the host because it reveals the presence of quiescent virus in cells. The latent viral load is useful in assessing a patient's disease status and the efficacy of highly active antiretroviral therapy and other treatment protocols.

CLM What is claimed is:

1. A method of determining the latent viral load in a host infected with **HIV** comprising, treating resting lymphoid mononuclear cells obtained from the host with an effective amount of an agent capable of activating an **HIV** virus integrated into the genome of the cells; and detecting the expression of cell-surface **gp120** after the cells have been treated with the agent, wherein the presence or amount of cells expressing cell-surface **gp120** is a measure of latent viral load.

2. A method of claims 1, further comprising obtaining the resting lymphoid mononuclear cells by the steps of: a) obtaining a sample cell population; b) depleting the sample cell population of cells expressing cell-surface **gp120**; and c) depleting sample cell population of cells expressing HLA-DR.

3. A method of claim 2, wherein the sample cells are depleted of **gp120** expressing cells by the steps of: a) contacting sample cells with **gp120**-specific **antibodies**, each conjugated to a capture moiety, under conditions effective for the **antibodies** to attach to **gp120** on the surface of cells, thereby forming labeled-cells; b) contacting the labeled-cells with capture moiety-specific **antibody** under conditions effective for the capture moiety-specific **antibody** to attach to the labeled-cells, thereby forming a complex-labeled cells; and c) removing the complex-labeled cells, thereby depleting sample cells of **gp120+** cells.

4. A method of claim 3, wherein the capture moiety-specific **antibody** is conjugated to magnetic particles.

5. A method of claim 3, wherein the capture moiety is FITC and the capture moiety-specific **antibody** is FITC-specific **antibody** conjugated to a magnetic bead.

6. A method of claims 4, wherein the magnetic particles are 10-100 nm in diameter.

7. A method of claims 5, wherein the magnetic particles are 10-100 nm in diameter.

8. A method of claims 3, wherein the removing is accomplished by a

magnetic field acting on the magnetic particles.

9. A method of claim 2, further comprising: separating **CD4**+ cells from the sample.

10. A method of claim 2, further comprising: separating CD8+ cells from the sample.

11. A method of claim 2, wherein the depleting sample cell population of cells expressing HLA-DR is accomplished by flow cytometry cell sorting and said cells are labeled with a fluorochrome-labeled **antibody** specific-for HLA-DR.

12. A method of claim 1, wherein the tissue is lymphoid.

13. A method of claims 1, wherein the agent is phorbol ester or a cytokine.

14. A method of claim 1, wherein the measure of latent viral load is number of cells expressing **gp120** after treating the resting with an effective amount of an agent capable of activating an **HIV** virus integrated into the genome of the cells.

15. A method of claim 1, wherein the measure of latent viral load is compared to an established cell line harboring latent **HIV**-1.

16. A method of claim 15, wherein the cell line is OM-10.1, U1, or Jurkat cells.

17. A method of treating a viral infection comprising measuring the latent viral load in an **HIV**-infected patient; and determining whether to administer to the patient an agent capable of activating an **HIV** virus integrated into the genome of a cell by the value of the latent viral load.

L15 ANSWER 15 OF 19 USPATFULL on STN
2001:114495 REAGENT SYSTEM AND KIT FOR DETECTING **HIV** INFECTED CELLS.
KING, CHESTER F., FREDERICK, MD, United States
HALLOWITZ, ROBERT A., GAITHERSBURG, MD, United States
US 2001008760 A1 20010719
APPLICATION: US 1998-139663 A1 19980825 (9) <--
WO 1997-US18649 19971015 None PCT 102(e) date
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to blood collection and diagnostics. More particularly, the invention relates to blood collection and diagnostics utilizing techniques such as magnetic separation and photodetection. The present invention also relates to methods and an apparatus for detecting the presence of antigens displayed on the surface of cells. More preferably, the present invention relates to the detection of cells infected by **human immunodeficiency virus** (**HIV**) and related viruses. In accordance with the present invention, **HIV**-infected cells can be detected and separated from uninfected cells. In a preferred embodiment, separation is achieved by a magnetic field. By coating the infected cells with magnetic particles, transfer of the cells to a precise location is facilitated. A novel aspect of the present invention is a cartridge antigen test which allows for the collection and mixing of blood with reagents in one package, which can be viewed on a fluorescent microscope.

CLM What is claimed is:
1. A method of separating cells expressing a viral antigen, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the mixture, a first binding partner specific for an antigen coded for by the virus which is

expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to the viral antigen on the cell surface; c) adding to the mixture resulting from b), a second binding partner specific for the first binding partner and attached to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and d) separating target cells containing the complex, whereby said separation is achieved by a magnetic field.

2. A method of claim 1, further comprising adding to the target cell a sample **antibody** specific for the viral antigen.

3. A method of claim 2, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample **antibody**

4. A method of claim 1, further comprising adding to the target cell a sample comprising an **antibody** specific for the viral antigen, whereby the amount of the second **antibody** is effective for interfering with the binding of the first binding partner to the viral antigen.

5. A method of claim 1, further comprising adding to the target cell a sample suspected of containing an **antibody** specific for the viral antigen.

6. A method of claim 5, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample.

7. A method of claim 6, wherein the first binding partner is an **antibody** specific for the viral antigen.

8. A method of claim 6, wherein the second binding partner is an **antibody** specific for the first binding partner.

9. A method of claim 6, wherein the first binding partner is an **antibody** specific for the viral antigen, which **antibody** is labeled with a detectable label.

10. A method of claim 9, wherein the second binding partner is an **antibody** specific for the detectable label.

11. A method of claim 6, wherein the first binding partner is an **antibody** specific for the viral antigen, which **antibody** is labeled with a detectable label.

12. A method of claim 6, wherein the virus is **HIV**.

13. A method of claim 6, wherein the first binding partner is an **antibody** specific for the viral antigen **gp120**, which **antibody** is labeled with a detectable label; and the second binding partner is an **antibody** specific for the detectable label.

14. A method of claim 6, wherein the target cell is a T-cell line.

15. A method of claim 6, wherein the sample is a body fluid or blood.

16. A method of claim 6, wherein measurement of the number of target cells separated in d) in the presence and absence of the sample is accomplished by flow cytometry.

17. A method of claim 12, wherein the first binding partner is a receptor for the viral antigen.

18. A method of claim 16, wherein the first binding partner is a receptor for the viral antigen and is labeled with a detectable label;

and the second binding partner is an **antibody** specific for the detectable label.

19. A method of claim 6, wherein the bead diameter is about 50-120 nm.

20. A method of claim 6, wherein the cell is contacted by at least about 100-1000 beads.

21. A method of identifying an agent which interferes with viral infection of a cell, a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test sample containing an agent suspected with interfering with viral infection of the test cell; c) adding to the mixture of b), a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen on the cell surface; d) adding to the resultant mixture formed in c), a second binding partner specific for the first binding partner and to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; e) separating test cells containing said complex, whereby said separation is achieved by a magnetic field; and f) determining the number of cells infected with said virus in the presence and the absence of said test agent.

22. A magnetic bead having a surface coated by a cell-surface virus receptor for **HIV**.

23. A magnetic bead of claim 21, wherein the virus receptor is **CD4**.

24. A method of separating virus-infected cells from non-virus infected cells in a sample comprising, combining (a) a first **antibody** recognizing a viral antigen on the surface of said cell and attached to a magnetic particle; (b) a second **antibody** recognizing said viral antigen on the surface of said cell and attached to a detectable label; and (c) a sample containing said virus-infected cells, to form a mixture; incubating said mixture under conditions effective for binding of said **antibodies** to said viral antigen to form a complex, said complex comprising said first and second **antibody** bound to said virus-infected cell, and moving said magnetic particle to a predetermined point on a reaction vessel holding said mixture, whereby said moving is accomplished by a magnetic field acting on said magnetic particle resulting in separating said virus-infected cells from non-virus infected cells, wherein said moving is accomplished without removing unbound **antibody** first and second **antibody** from said mixture.

25. A method of claim 24, further comprising detecting the label of said second **antibody** bound to said viral antigen on said virus-infected cell, wherein said first and second **antibody** recognize different epitopes of said viral antigen.

26. A method of separating cells infected with a virus, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus; b) fixing and permeabilizing said cells; c) adding to the fixed and permeabilized cells, a first binding partner specific for an antigen coded for by the virus, which viral antigen is ultimately expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to said viral antigen on the inside of said fixed and permeabilized cell; d) adding to the result of c), a second binding partner specific for the first binding partner and attached to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a

complex; and e) separating target cells containing the complex, whereby said separation is achieved by a magnetic field.

27. A method of identifying an agent which interferes with viral infection of a cell, comprising: a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test agent suspected with interfering with viral infection of the test cell; c) fixing and permeabilizing said cells; d) adding a first binding partner specific for an antigen coded for by the virus, which viral antigen is expressed ultimately on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen when said viral antigen is expressed in the interior of said cell; e) adding to the resultant mixture formed in d), a second binding partner specific for the first binding partner and to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; f) separating test cells containing said complex, whereby said separation is achieved by a magnetic field; and g) determining whether the test sample changes the number of test cells containing the complex when compared to the process performed in the absence of said agent.

28. A method claim 27, where said test agent is added to cells prior to simultaneous to contacting cell with said test agent.

29. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen **antibody** attached to a detectable label, an effective amount of an **antibody** specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture, wherein said **antibody** specific-for said detectable label is attached to a magnetic particle; b) incubating said mixture under conditions effective for binding of said anti-cell surface viral **antibody** to said cell-surface viral antigen, and, for binding of said **antibody** specific-for said detectable label to said detectable label attached to said anti-cell surface viral **antibody**, to form a complex, wherein said anti-viral **antibody** is bound to said cell-surface antigen displayed on a viral-infected cell; and c) separating said complex, comprising said cells expressing said cell-surface viral antigen and magnetic particles, by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field.

30. A method of claim 29, wherein viral-infected cells are infected with **HIV**.

31. A method of claim 29, wherein said cell-surface viral antigen is an envelope glycoprotein for **HIV**.

32. A method of claim 29, wherein the envelope glycoprotein is **gp120** or gp41.

33. A method of claim 29, wherein said anti-cell surface viral **antibody** is a polyclonal **antibody** specific for **HIV** envelope glycoprotein and said viral-infected cells are infected with **HIV**.

34. A method of claim 29, wherein said detectable label is FITC, TRITC, or R-phycoerthryin.

35. A method of claim 29, further comprising counting said magnetically-separated cells by flow cytometry.

36. A method of claim 29, wherein said magnetic particles are about 10-150 nm in diameter. A method of separating cells expressing a

cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen **antibody** attached to a magnetic particle and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture; b) incubating said mixture under conditions effective for binding of said anti-cell surface viral **antibody** to said cell-surface viral antigen displayed on said viral-infected cells, to form a complex; and c) separating said complex comprising said cells expressing said cell-surface viral antigen and magnetic particles by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field.

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=> d his

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                E KROWKA JOHN/IN
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L3              2 S E3 OR E4
L4          33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5           3422 S L4 AND (GP120 OR GP160)
L6           2490 S L5 AND (CD4?)
L7            182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8            182 S L7 AND ANTIBOD?
L9             16 S L8 AND (GP120/CLM OR GP160/CLM)
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FILE 'MEDLINE' ENTERED AT 14:21:56 ON 17 JUN 2004

 FILE LAST UPDATED: 16 JUN 2004 (20040616/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

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L16           3 "HALLOWITZ R A"/AU

=> d l16,cbib,ab,1-3
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L16 ANSWER 1 OF 3 MEDLINE on STN
77223116. PubMed ID: 195672. Effects of vagal volleys on units of intralaminar and juxtalaminar thalamic nuclei in monkeys. **Hallowitz R A**; MacLean P D. Brain research, (1977 Jul 15) 130 (2) 271-86. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.
AB As part of an attempt to clarify the nature of inputs to the limbic cortex, the thalamic intralaminar and juxtalaminar nuclei were explored for unit responses to vagal volleys in awake, sitting squirrel monkeys. Vagal shocks elicited responses of a large percentage of units in the anterior medial, paracentral, lateral dorsal, and lateral, and medial dorsal nuclei, as well as in part of the ventral lateral nucleus adjacent to the paracentral. Responsive units showed either initial excitation or initial inhibition. As in the preceding study on the cingulate and supracingulate cortex, there were two main types of initially excited units: type 1 responded with a discharge of 1-3 spikes at relatively short and constant latencies, while type 2 units were characterized by a burst of 3-14 spikes at longer and more variable latencies. Although the findings were compatible with the hypothesis that the anterior and paracentral nuclei transmit vagal impulses to the cingulate and supracingulate cortex, an analysis of latencies suggested that a more rapidly conducting pathway(s) accounts for latencies as short as 12 msec of some cingulate units. Twenty-eight percent of 367 units in the medial dorsal nucleus responded to vagal volleys. This finding gives substantial support to the traditional view that the medial dorsal nucleus transmits interoceptive information to limbic and neocortical areas of the orbitofrontal region.

L16 ANSWER 2 OF 3 MEDLINE on STN
77223115. PubMed ID: 406970. Effects of vagal volleys and serotonin on units of cingulate cortex in monkeys. Bachman D S; **Hallowitz R A**; MacLean P D. Brain research, (1977 Jul 15) 130 (2) 253-69. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

L16 ANSWER 3 OF 3 MEDLINE on STN
72084030. PubMed ID: 4400030. Forebrain activation of single units in preoptic area of sunfish. **Hallowitz R A**; Woodward D J; Demski L S. Comparative biochemistry and physiology. A, Comparative physiology, (1971 Nov 1) 40 (3) 733-41. Journal code: 1276312. ISSN: 0300-9629. Pub. country: United States. Language: English.

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=> s e2
L17           1 "HALLOWITZ R"/AU

=> d l17,cbib,ab
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L17 ANSWER 1 OF 1 MEDLINE on STN
2001080085. PubMed ID: 11073782. Limitations of plasma human immunodeficiency virus RNA testing. Krowka J F; Sheppard H W; Ascher M S; **Hallowitz R**. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, (2000 Nov) 31 (5) 1317-8. Journal code: 9203213. ISSN: 1058-4838. Pub. country: United States. Language: English.

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=> file biosis
COST IN U.S. DOLLARS                              SINCE FILE     TOTAL
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FULL ESTIMATED COST                                     2.25    170.01

FILE 'BIOSIS' ENTERED AT 14:24:22 ON 17 JUN 2004
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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 16 June 2004 (20040616/ED)

FILE RELOADED: 19 October 2003.

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=> s e3 or e4
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               1 "HALLOWITZ ROBERT A"/AU
L18            8 "HALLOWITZ R A"/AU OR "HALLOWITZ ROBERT A"/AU

=> d l18,ti,1-8

L18  ANSWER 1 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Methods of improving infectivity of cells for viruses.

L18  ANSWER 2 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Reagent system for detecting HIV-infected peripheral blood lymphocytes in
     whole blood.

L18  ANSWER 3 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Cartridge test system fort he collection and testing of blood in a single
     step.

L18  ANSWER 4 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Four drugs are better than three drugs to maintain existing HIV
     suppression and reduce productive infection.

L18  ANSWER 5 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   EFFECTS OF VAGAL VOLLEYS ON UNITS OF INTRA LAMINAR AND JUXTALAMINAR
     THALAMIC NUCLEI IN MONKEYS.

L18  ANSWER 6 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   EFFECTS OF VAGAL VOLLEYS AND SEROTONIN ON UNITS OF CINGULATE CORTEX IN
     MONKEYS.

L18  ANSWER 7 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   EFFECTS OF VAGAL VOLLEYS ON UNIT ACTIVITY OF MEDIAL THALAMIC NUCLEI IN
     SQUIRREL MONKEYS SAIMIRI-SCIUREUS.

L18  ANSWER 8 OF 8  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   FORE BRAIN ACTIVATION OF SINGLE UNITS IN PREOPTIC AREA OF SUNFISH.
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=> d l18,cbib,ab,1-4

L18 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:611058 Document No.: PREV200200611058. Methods of improving infectivity of cells for viruses. **Hallowitz, Robert A.** [Inventor, Reprint author]; Young, Susan [Inventor]; King, Chester [Inventor]. Gaithersburg, MD, USA. ASSIGNEE: Bio-Tech Imaging, INC, Frederick, MD, USA. Patent Info.: US 6461809 October 08, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 8, 2002) Vol. 1263, No. 2. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB The present invention relates to cells which have improved receptivity to viruses which are capable of infecting them. Receptivity to such viruses is improved by selecting cells from a population which express the receptor(s) that enable a virus to attach to the cell and gain entry into it. Any combination of viruses and host cell lines can be used. In a preferred embodiment, the present invention relates to improving receptivity or infectivity of a cell line which can be infected with an immunodeficiency virus, such as HIV-1. Especially preferred embodiments of the invention relate to methods of improving (or assaying for) the infectivity for HIV-1 in a HIV-1 receptive cell line, preferably a continuous cell line transformed with DNAs coding for expressible CD4 and expressible HIV-1 coreceptor, comprising, in any effective order, a) isolating the cells expressing CD4 and an HIV-1 coreceptor on their cell surface; b) contacting the isolated cells with HIV-1 under conditions effective for the HIV-1 to infect the cells; and c) detecting the number of cells infected with HIV-1, thereby assaying for infectivity of HIV-1. This method facilitates the measurement of true infectivity and infectivity reduction values by quantifying the percentage of infected cells in the population of specific cells capable of being infected by virus, rather than in a population of mixed cells, only some which are capable of being infected.

L18 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:129073 Document No.: PREV200200129073. Reagent system for detecting HIV-infected peripheral blood lymphocytes in whole blood. King, C. F. [Inventor]; **Hallowitz, R. A.** [Inventor]. Frederick, Md., USA. ASSIGNEE: THE AVRIEL GROUP, AMCAS DIVISION INC.. Patent Info.: US 5817458 Oct. 6, 1998. Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 6, 1998) Vol. 1215, No. 1, pp. 532. print. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

L18 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:102751 Document No.: PREV200200102751. Cartridge test system fort he collection and testing of blood in a single step. **Hallowitz, R. A.** [Inventor]; King, C. F. [Inventor]. Montgomery, Md., USA. ASSIGNEE: BIO-TECH IMAGING, INC.. Patent Info.: US 5714390 Feb. 3, 1998. Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 3, 1998) Vol. 1207, No. 1, pp. 422. print. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

L18 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:8835 Document No.: PREV200100008835. Four drugs are better than three drugs to maintain existing HIV suppression and reduce productive infection. Fessel, W. J. [Reprint author]; Anderson, B. [Reprint author]; Follansbee, S. E. [Reprint author]; Luu, T. T. [Reprint author]; Young, T. P. [Reprint author]; Salas, V. M.; **Hallowitz, R. A.**; Silberman, S. R.; White, J. M. [Reprint author]. Kaiser Permanente, San Francisco, CA, USA. Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2000) Vol. 40, pp. 279. print. Meeting Info.: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto, Ontario, Canada. September 17-20, 2000. Interscience Conference on Antimicrobial Agents and Chemotherapy; American Society of Microbiology.

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Language: English.


=> file medline
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FULL ESTIMATED COST                                     9.75     179.76

FILE 'MEDLINE' ENTERED AT 14:25:54 ON 17 JUN 2004

 FILE LAST UPDATED: 16 JUN 2004 (20040616/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

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E10            1      KROWLIKOWSKI A/AU
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E12            1      KROWLL I/AU

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L19            9 "KROWKA J"/AU

=> d l19,ti,1-9

L19  ANSWER 1 OF 9     MEDLINE on STN
TI   CD4+ T cell surface CCR5 density and virus load in persons infected with
     human immunodeficiency virus type 1.

L19  ANSWER 2 OF 9     MEDLINE on STN
TI   PCR-Based assay to quantify human immunodeficiency virus type 1 DNA in
     peripheral blood mononuclear cells.

L19  ANSWER 3 OF 9     MEDLINE on STN
TI   Cross-clade human immunodeficiency virus (HIV)-specific cytotoxic
     T-lymphocyte responses in HIV-infected Zambians.

L19  ANSWER 4 OF 9     MEDLINE on STN
TI   Molluscum contagiosum virus grows in human skin xenografts.

L19  ANSWER 5 OF 9     MEDLINE on STN
TI   The SCID-hu mouse: a small animal model for HIV infection and
     pathogenesis.

L19  ANSWER 6 OF 9     MEDLINE on STN
TI   Monocyte-mediated lysis of HIV-infected tumor cells.

L19  ANSWER 7 OF 9     MEDLINE on STN
TI   Lymphocyte proliferative responses to soluble and liposome-conjugated
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     envelope peptides of HIV-1.

L19  ANSWER 8 OF 9     MEDLINE on STN
TI   Long-term observation of baboons, rhesus monkeys, and chimpanzees
     inoculated with HIV and given periodic immunosuppressive treatment.

L19  ANSWER 9 OF 9     MEDLINE on STN
TI   Effects of interleukin 2 and large envelope glycoprotein (gp 120) of human
     immunodeficiency virus (HIV) on lymphocyte proliferative responses to
     cytomegalovirus.


=> file uspatful
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FULL ESTIMATED COST                                       0.76     180.52

FILE 'USPATFULL' ENTERED AT 14:27:23 ON 17 JUN 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 17 Jun 2004 (20040617/PD)
FILE LAST UPDATED: 17 Jun 2004 (20040617/ED)
HIGHEST GRANTED PATENT NUMBER: US6751803
HIGHEST APPLICATION PUBLICATION NUMBER: US2004117887
CA INDEXING IS CURRENT THROUGH 17 Jun 2004 (20040617/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 17 Jun 2004 (20040617/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Apr 2004
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Apr 2004

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>>>  published document but also a list of any subsequent               <<<
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     (FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
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L1            7 S E4 OR E5
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L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
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L7           182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8           182 S L7 AND ANTIBOD?
L9            16 S L8 AND (GP120/CLM OR GP160/CLM)
L10           20 S L7 AND AY<2000
L11           15 S L10 NOT L9
L12         2375 S L6 AND ANTIBOD?
L13         1113 S L12 AND (ANTIBOD?/CLM)
L14          211 S L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERA
L15           19 S L14 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 14:21:56 ON 17 JUN 2004
                E HALLOWITZ R A/AU
L16            3 S E3
L17            1 S E2

     FILE 'BIOSIS' ENTERED AT 14:24:22 ON 17 JUN 2004
                E HALLOWITZ R A/AU
L18            8 S E3 OR E4

     FILE 'MEDLINE' ENTERED AT 14:25:54 ON 17 JUN 2004
                E KROWKA J/AU
L19            9 S E3

     FILE 'USPATFULL' ENTERED AT 14:27:23 ON 17 JUN 2004

=> s l6 and (paramagnetic or magnetic)
          10105 PARAMAGNETIC
         470699 MAGNETIC
L20          800 L6 AND (PARAMAGNETIC OR MAGNETIC)

=> s l20 and (magnetic/clm or paramagnetic/clm)
         133855 MAGNETIC/CLM
           1743 PARAMAGNETIC/CLM
L21           39 L20 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)

=> s l21 and ay<2001
        3177390 AY<2001
L22           19 L21 AND AY<2001

=> d l22,ti,1-19

L22  ANSWER 1 OF 19  USPATFULL on STN
TI     Humanized and chimeric N-terminal amyloid beta-antibodies

L22  ANSWER 2 OF 19  USPATFULL on STN
TI     Determining viral load in double negative T cells

L22  ANSWER 3 OF 19  USPATFULL on STN
TI     Method for activity profiling compound mixtures

L22  ANSWER 4 OF 19  USPATFULL on STN
TI     Up-converting reporters for biological and other assays using laser
       excitation techniques

L22  ANSWER 5 OF 19  USPATFULL on STN
TI     Method for electromagnetically restructuring water for organismic
       consumption

L22  ANSWER 6 OF 19  USPATFULL on STN
TI     Cyanovirin conjugates and matrix-anchored cyanovirin and related
       compositions and methods of use

L22  ANSWER 7 OF 19  USPATFULL on STN
TI     Up-converting reporters for biological and other assays using laser
       excitation techniques
```

L22 ANSWER 8 OF 19 USPATFULL on STN
TI METHODS OF IMPROVING INFECTIVITY OF CELLS FOR VIRUSES

L22 ANSWER 9 OF 19 USPATFULL on STN
TI Consensus configurational bias Monte Carlo method and system for pharmacophore structure determination

L22 ANSWER 10 OF 19 USPATFULL on STN
TI METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD

L22 ANSWER 11 OF 19 USPATFULL on STN
TI REAGENT SYSTEM AND KIT FOR DETECTING **HIV** INFECTED CELLS

L22 ANSWER 12 OF 19 USPATFULL on STN
TI Synthetic human neutralizing monoclonal antibodies to **human immunodeficiency virus**

L22 ANSWER 13 OF 19 USPATFULL on STN
TI Method of using solid state NMR to measure distances between nuclei in compounds attached to a surface

L22 ANSWER 14 OF 19 USPATFULL on STN
TI Reagent system for detecting **HIV**-infected peripheral blood lymphocytes in whole blood

L22 ANSWER 15 OF 19 USPATFULL on STN
TI Methods for screening of test compounds for inhibiting binding of a **CD4-HIV** 1 complex to a chemokine receptor

L22 ANSWER 16 OF 19 USPATFULL on STN
TI Up-converting reporters for biological and other assays using laser excitation techniques

L22 ANSWER 17 OF 19 USPATFULL on STN
TI Up-converting reporters for biological and other assays using laser excitation techniques

L22 ANSWER 18 OF 19 USPATFULL on STN
TI Anti-viral therapeutic composition

L22 ANSWER 19 OF 19 USPATFULL on STN
TI Method for separating pathogenic or toxic agents from a body fluid and return to body

=> d l22,cbib,ab,clm,17,16,14,12,11,10

L22 ANSWER 17 OF 19 USPATFULL on STN
97:91360 Up-converting reporters for biological and other assays using laser excitation techniques.
Zarling, David A., Menlo Park, CA, United States
Rossi, Michel J., Lausanne, Switzerland
Peppers, Norman A., Belmont, CA, United States
Kane, James, Lawrenceville, NJ, United States
Faris, Gregory W., Menlo Park, CA, United States
Dyer, Mark J., San Jose, CA, United States
Ng, Steve Y., San Francisco, CA, United States
Schneider, Luke V., Half Moon Bay, CA, United States
SRI International, Menlo Park, CA, United States (U.S. corporation)
US 5674698 19971007
APPLICATION: US 1995-416023 19950330 (8) <--
PRIORITY: WO 1993-US8712 19930914
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods, compositions, and apparatus for performing sensitive detection of analytes, such as biological

macromolecules and other analytes, by labeling a probe molecule with an up-converting label. The up-converting label absorbs radiation from an illumination source and emits radiation at one or more higher frequencies, providing enhanced signal-to-noise ratio and the essential elimination of background sample autofluorescence. The methods, compositions, and apparatus are suitable for the sensitive detection of multiple analytes and for various clinical and environmental sampling techniques.

CLM What is claimed is:

1. A method for detecting an analyte in a sample, comprising the steps of: contacting a sample containing a target analyte with a labeled binding component to specifically bind the target analyte and form a labeled binding component-target complex, wherein the labeled binding component comprises a binding component attached to an up-converting inorganic phosphor particle comprising at least one rare earth element and a phosphor host material and being capable of converting excitation radiation to emission radiation of a shorter wavelength; separating any unbound labeled binding component from the labeled binding component-target complex; illuminating the labeled binding component-target complex with excitation radiation; and detecting emission radiation of at least one label emission wavelength, wherein the emission radiation has a shorter wavelength than the excitation radiation.

2. A method according to claim 1, wherein said up-converting inorganic phosphor comprises ytterbium and erbium in a phosphor host material.

3. A method according to claim 2, wherein the up-converting inorganic phosphor comprises sodium yttrium fluoride ytterbium erbium or yttrium ytterbium erbium oxysulfide.

4. A method of claim 1, further comprising, before the contacting step, the step of attaching the up-converting inorganic phosphor particle to the binding component to form the labeled binding component.

5. A method according to claim 4, wherein the binding component is attached to the label by covalent or noncovalent binding.

6. A method according to claim 5, wherein the binding component is streptavidin or avidin and the target analyte is a biotinylated target analyte.

7. A method of claim 1, wherein the labeled binding component-target complex is separated from the unbound labeled probe by immobilization on a solid support.

8. A method according to claim 7, wherein the step of separating unbound labeled binding component from the labeled binding component-target complex in the sample is performed by washing the sample with an aqueous solution to remove suspendible or soluble unbound labeled binding component.

9. A method of claim 7, wherein the labeled binding component target complex is bound to a first binding component on the solid support to form a sandwich complex.

10. A method according to claim 1, wherein the target analyte is selected from the group consisting of: polynucleotides, polypeptides, viruses, microorganisms, haptens, mammalian cells, steroid hormones, glycoproteins, lipoproteins, biotinylated **magnetic** beads, prescribed or over-the-counter drugs, illegal substances, intoxicants and drugs of abuse.

11. A method according to claim 1, wherein the step of illuminating with a label excitation wavelength is performed with an infrared laser diode or light-emitting diode.

12. A method according to claim 1, wherein the infrared laser diode or light-emitting diode emits pulsed illumination.

13. A method according to claim 12, wherein the infrared laser diode or light-emitting diode is pulsed through direct current modulation.

14. A method according to claim 12, wherein the step of detecting light emission of at least one label emission wavelength is performed by time-gated or lock-in detection.

15. A method according to claim 11, wherein the step of detecting light emission is performed with phase-sensitive detection.

16. A method according to claim 11, wherein the laser diode or light-emitting diode has peak emissions in the range of 960-980 nm and at approximately 1500 nm.

17. A method according to claim 1, wherein said step of detecting light emission is performed with a photomultiplier, photodiode, a charge coupled device, a charge injection device, or photographic film emulsion.

18. A method According to claim 1, wherein the target analyte is immobilized in a histological tissue section or a solid support.

19. A method according to claim 1, wherein the binding component is selected from the group consisting of: antibodies, polynucleotides, polypeptide hormones, streptavidin, Staphylococcus aureus Protein A, lectins, and antigens.

20. A method for detecting an analyte in a sample, comprising the steps of: contacting a sample containing a target analyte with a binding component to specifically bind the target analyte and form a binding component-target complex; contacting the binding component-target complex with a label to form a labeled binding component-target complex, the label comprising an up-converting inorganic phosphor particle comprising at least one rare earth element and a phosphor host material and being capable of converting excitation radiation to emission radiation of a shorter wavelength; separating any unbound label from the labeled binding component-target complex; illuminating the labeled binding component-target complex with excitation radiation; and detecting emission radiation of at least one label emission wavelength, wherein the emission radiation has a shorter wavelength than the excitation radiation.

21. A method according to claim 20, wherein the binding component is a primary antibody and the up-converting inorganic phosphor is bound to the binding component through a secondary antibody.

22. A method according to claim 21, wherein said secondary antibody is biotinylated and said up-converting inorganic phosphor is bound to streptavidin.

23. A method according to claim 20, wherein the target analyte is a polynucleotide and the binding component is a biotinylated polynucleotide which hybridizes to the target polynucleotide under binding conditions.

24. A method according to claim 23, wherein the up-converting inorganic phosphor comprises an up-converting phosphor particle and streptavidin.

25. A method of claim 20 further comprising, before the contacting step, the step of attaching the up-converting inorganic phosphor particle to the binding component to form the labeled binding component.

26. A method for detecting a biotinylated analyte in a sample, comprising the steps of: contacting a sample containing a biotinylated analyte with a labelled binding component to specifically bind the biotinylated analyte and form a labeled binding component-target complex, wherein the labeled binding component comprises a streptavidin-coated up-converting inorganic phosphor particle, the up-converting inorganic phosphor particle comprising at least one rare earth element and a phosphor host material and being capable of converting excitation radiation to emission radiation of a shorter wavelength; separating any unbound labeled binding component from the labeled binding component-target complex; illuminating the labeled binding component-target complex with excitation radiation; and detecting emission radiation of at least one label emission wavelength, wherein the emission radiation has a shorter wavelength than the excitation radiation.

27. A method according to claim 26, wherein the target is a biotinylated **magnetic** bead.

28. A method of claim 26 further comprising, before the contacting step, the step of attaching the up-converting inorganic phosphor particle to the binding component to form the labeled binding component.

29. A method for detecting an analyte in a sample, comprising the steps of: contacting a sample containing a target analyte with a labeled binding component to specifically bind the target analyte and form a labeled binding component-target complex, wherein the labeled binding component comprises a binding component attached to an up-converting inorganic phosphor particle comprising at least one rare earth element and a phosphor host material and being capable of converting excitation radiation to emission radiation of a shorter wavelength; differentiating the labeled binding component-target complex from any unbound labeled binding component in the sample; illuminating the labeled binding component-target complex with excitation radiation; and detecting emission radiation of at least one label emission wavelength from the labeled binding component-target complex, wherein the emission radiation has a shorter wavelength than the excitation radiation.

30. The method of claim 29, wherein the labeled binding component-target complex is illuminated with a confocal beam having a focal point at the contact surface and being divergent at points other than the contact surface.

31. A method of claim 29, further comprising, before the contacting step, the step of attaching the up-converting inorganic phosphor particle to the binding component to form the labeled binding component.

32. A method of claim 29, wherein the differentiating step comprises contacting the labeled binding component-target complex with a contact surface wherein the labeled binding component-target complex is localized at the contact surface as compared to the unbound labeled binding component; and the illuminating step comprises illuminating the labeled binding component-target complex at the contact surface with excitation radiation.

33. The method of claim 32, wherein binding component-target complexes are localized to the contact surface by a method selected from the group consisting of: **magnetic** localization of **magnetic** beads to said contact surface, wherein said binding component-target complexes are localized on the **magnetic** beads relative to unbound labeled binding component; gravitational sedimentation of binding component-target complexes from unbound labelled binding component, wherein said sedimented binding component-target complexes are localized on the contact surface relative to unbound labeled binding component; filtration over a contact surface wherein said binding component-target complexes are localized on the contact surface relative to unbound

labeled binding component; antibody capture; affinity adsorption; and nucleic acid hybridization.

34. A method of claim 29, wherein the differentiating and illuminating steps are accomplishied by confocal excitation.

35. A method of claim 29 wherein the differentiating, illuminating, and detecting steps are accomplished by confocal excitation and confocal detection.

36. A method of claim 29, wherein the differentiating step is accomplished by size discrimination.

L22 ANSWER 16 OF 19 USPATFULL on STN
97:117891 Up-converting reporters for biological and other assays using laser excitation techniques.
Zarling, David A., Menlo Park, CA, United States
Rossi, Michel J., Lausanne, Switzerland
Peppers, Norman A., Belmont, CA, United States
Kane, James, Lawrenceville, NJ, United States
Faris, Gregory W., Menlo Park, CA, United States
Dyer, Mark J., San Jose, CA, United States
Ng, Steve Y., San Francisco, CA, United States
Schneider, Luke V., Half Moon Bay, CA, United States
SRI International, Menlo Park, CA, United States (U.S. corporation)
US 5698397 19971216
APPLICATION: US 1995-482203 19950607 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods, compositions, and apparatus for performing sensitive detection of analytes, such as biological macromolecules and other analytes, by labeling a probe molecule with an up-converting label. The up-converting label absorbs radiation from an illumination source and emits radiation at one or more higher frequencies, providing enhanced signal-to-noise ratio and the essential elimination of background sample autofluorescence. The methods, compositions, and apparatus are suitable for the sensitive detection of multiple analytes and for various clinical and environmental sampling techniques.

CLM What is claimed is:
1. A composition comprising an up-converting inorganic phosphor which converts excitation radiation to emission radiation of a shorter wavelength and comprises at least one rare earth element in a host material and a probe selected from the group consisting of: antibodies, avidins, lectins, Staphylococcus aureus Protein A, antigens, polypeptides and polynucleotides.

2. A composition according to claim 1, wherein the up-converting inorganic phosphor comprises ytterbium and an emitter selected from erbium, holmium, thulium, and terbium.

3. A composition according to claim 1, wherein the probe is attached to an up-converting inorganic phosphor by noncovalent linkage.

4. A composition according to claim 1, wherein the probe is streptavidin and the up-converting inorganic phosphor is attached to the probe by noncovalent linkage.

5. A composition of claim 4 further comprising a biotinylated **magnetic** bead, a streptavidin-coated **magnetic** bead, an avidin-coated **magnetic** bead, or an immunoglobulin-coated **magnetic** bead.

6. A composition for diagnostic detection of an analyte, comprising a microcrystalline up-convening phosphor covalently bound to antibodies, avidins, lectins, Staphylococcus aureus Protein A. antigens,

polypeptides and polynucleotides.

7. A composition of claim 6, wherein the microcrystalline up-converting phosphor has the formula: $Na(Y_x Yb_y Er_z)F_4$: wherein x is 0.7 to 0.9, y is 0.09 to 0.29, and z is 0.05 to 0.01; $Na(Y_x Yb_y Ho_z)F_4$: wherein x is 0.7 to 0.9, y is 0.0995 to 0.2995, and z is 0.0005 to 0.001; $Na(Y_x Yb_y Tm_z)F_4$: wherein x is 0.7 to 0.9, y is 0.0995 to 0.2995, and z is 0.0005 to 0.001; or $(Y_x Yb_y Er_z)O_2 S$: wherein x is 0.7 to 0.9, y is 0.05 to 0.12; z is 0.05 to 0.12.

8. A composition of claim 6, wherein the microcrystalline up-converting phosphor has a formula selected from the group consisting of: $(Y_0.80\ Yb_0.18\ Er_0.02\ F_3$; $(Y_0.87\ Yb_0.13\ Tm_0.001)F_3$; $(Y_0.80\ Yb_0.198\ Ho_0.002)F_3$; $(Gd_0.08\ Yb_0.18\ Er_0.02)F_3$; $(Gd_0.87\ Yb_0.13\ Tm_0.001)F_3$; $(Gd_0.80\ Yb_0.198\ Ho_0.002)F_3$; $(Y_0.86\ Yb_0.08\ Er_0.06)_2\ O_2\ S$; $(Y_0.87\ Yb_0.13\ Tm_0.001)_2\ O_2\ S$; $(Y_0.08\ Yb_0.198\ Ho_0.0022)O_2\ S$; $(Gd_0.86\ Yb_0.08\ Er_0.06)_2\ O_2\ S$; $(Gd_0.87\ Yb_0.13\ Tm_0.001)_2\ O_2\ S$; and $(Gd_0.08\ Yb_0.198\ Ho_0.002)_2\ O_2\ S$.

9. A composition for diagnostic detection of an analyte, comprising an up-converting phosphor non-covalently bound to antibodies, avidins, lectins, Staphylococcus aureus Protein A, antigens, polypeptides and polynucleotides.

10. A composition of claim 9 wherein the microcrystalline up-converting phosphor has the formula: $Na(Y_x Yb_y Er_z)F_4$: wherein x is 0.7 to 0.9, y is 0.09 to 0.29, and z is 0.05 to 0.01; $Na(Y_x Yb_y Ho_z)F_4$: wherein x is 0.7 to 0.9, y is 0.0995 to 0.2995, and z is 0.0005 to 0.001; $Na(Y_x Yb_y Tm_z)F_4$: wherein x is 0.7 to 0.9, y is 0.0995 to 0.2995, and z is 0.0005 to 0.001; or $(Y_x Yb_y Er_z)O_2 S$: wherein x is 0.7 to 0.9, y is 0.05 to 0.12; z is 0.05 to 0.12.

11. A composition of claim 9, wherein the microcrystalline up-converting phosphor has a formula selected from the group consisting of: $(Y_0.80\ Yb_0.18\ Er_0.02)F_3$; $(Y_0.87\ Yb_0.13\ Tm_0.001)F_3$; $(Y_0.80\ Yb_0.198\ Ho_0.002)F_3$; $(Gd_0.80\ Yb_0.18\ Er_0.02)F_3$; $(Gd_0.87\ Yb_0.13\ Tm_0.001)F_3$; $(Gd_0.80\ Yb_0.198\ Ho_0.002)F_3$; $(Y_0.86\ Yb_0.08\ Er_0.06)_2\ O_2\ S$; $(Y_0.87\ Yb_0.13\ Tm_0.001)_2\ O_2\ S$; $(Y_0.80\ Yb_0.198\ Ho_0.002)_2\ O_2\ S$; $(Gd_0.86\ Yb_0.08\ Er_0.06)_2\ O_2\ S$; $(Gd_0.87\ Yb_0.13\ Tm_0.001)_2\ O_2\ S$; and $(Gd_0.08\ Yb_0.198\ Ho_0.002)_2\ O_2\ S$.

L22 ANSWER 14 OF 19 USPATFULL on STN
1998:122214 Reagent system for detecting **HIV**-infected peripheral blood lymphocytes in whole blood.
King, Chester F., Frederick, MD, United States
Hallowitz, Robert A., Gaithersburg, MD, United States
The Avriel Group, AMCAS Division Inc., United States (part interest) a part interest
US 5817458 19981006
APPLICATION: US 1996-732782 19961015 (8) <--
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Fluorometric immunological assay method for detection of **HIV**-1 infection in which Murine anti-**gp120** monoclonal antibodies coupled to **paramagnetic** microspheres (14) and Fluorescein conjugated anti-**gp120** polyclonal antibodies IgG (16) are incubated in a few drops of whole blood diluted in 0.5 cc phosphate buffered saline (10). After incubation for 5 minutes, the **HIV**-infected peripheral blood lymphocytes (18) will be coated with both the Murine anti-**gp120** monoclonal antibodies coupled to **paramagnetic** microspheres (14) and Fluorescein conjugated anti-**gp120** polyclonal antibodies IgG (16) at exposed **gp120** antigens (20) binding sites. At the time of measurement said **HIV**- infected peripheral blood lymphocytes (18) will be pulled against the wall of the measurement vessel by means of a **magnetic** gradient (26). The cells adhering to the vessel wall are illuminated at 488 nm monochromatic light by a focused light source (28) and the resultant emitted fluorescence is imaged, measured and recorded.

CLM What is claimed is:

1. A method of detecting an **HIV**-infected cell in an aqueous sample comprising the steps of, a) combining a first anti-**gp120** antibody attached to a **magnetic** particle; a second anti-**gp120** antibody attached to a detectable label; and an aqueous sample containing **HIV**-infected peripheral blood lymphocytes displaying **gp120** on the cell surface, to form a mixture; b) incubating said mixture under conditions effective for binding of said antibodies to said **gp120** to form a complex, said complex comprising said first and second antibody bound to a **HIV**-infected cell on said **magnetic** particle; and c) moving said **magnetic** particle to a predetermined point on a reaction vessel holding said mixture, wherein said moving is accomplished by a **magnetic** field acting on said **magnetic** particle; d) detecting the label of said second antibody bound to **gp120** on said **HIV**-infected cell, with the proviso that no step of washing of said mixture and no step of removing unbound first antibody and unbound second antibody from said mixture is performed in steps a), b), c), and d).

2. A method of claim 1, wherein said first and second antibody recognize different regions of **gp120**.

3. A method of claim 1, wherein said aqueous sample is whole blood.

4. A method of claim 1, wherein said predetermined point is illuminated with a light effective to detect said label.

5. A method of claim 1, wherein said detectable label is FITC.

6. A method of claim 1, wherein said first antibody is a monoclonal antibody.

7. A method of claim 1, wherein said second antibody is a polyclonal antibody.

8. A method of claim 1, where said first antibody is a monoclonal antibody and said second antibody is a polyclonal antibody coupled to a detectable label which is FITC.

9. A method of detecting an **HIV**-infected cell in an aqueous sample comprising the steps of, a) combining a first anti-**gp120** antibody attached to a **magnetic** particle; a second anti-**gp120** antibody attached to a detectable label; and an aqueous sample containing **HIV**-infected cells displaying **gp120** on the cell surface, to form a mixture; b) incubating said mixture under conditions effective for binding of said antibodies to said **gp120** to form a complex, said complex comprising said first and second antibody bound to a **HIV**-infected cell on said **magnetic** particle; and c) moving said **magnetic** particle to a predetermined point on a reaction vessel holding said mixture, wherein said moving is accomplished by a **magnetic** field acting on said **magnetic** particle; d) detecting the

label of said second antibody bound to **gp120** on said **HIV**-infected cell, with the proviso that no step of washing of said mixture and no step of removing unbound first antibody and second antibody from said mixture is performed in a), b), c), and d).

10. A method of claim 1, wherein said **HIV**-infected cell is a peripheral blood lymphocyte.

11. A method of claim 9, wherein said first and second antibody recognize different regions of **gp120**.

12. A method of claim 9, wherein said aqueous sample is whole blood.

13. A method of claim 9, wherein said predetermined point is illuminated with a light effective to detect said label.

14. A method of claim 9, wherein said detectable label is FITC.

15. A method of claim 9, wherein said first antibody is a monoclonal antibody.

16. A method of claim 9, wherein said second antibody is a polygonal antibody.

17. A method of claim 9, where said first antibody is a monoclonal antibody and said second antibody is a polyclonal antibody coupled to a detectable label which is FITC.

L22 ANSWER 12 OF 19 USPATFULL on STN
2001:111829 Synthetic human neutralizing monoclonal antibodies to **human immunodeficiency virus**.
Barbas, Carlos F., San Diego, CA, United States
Burton, Dennis R., La Jolla, CA, United States
Lerner, Richard A., La Jolla, CA, United States
The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)
US 6261558 B1 20010717
WO 9511317 19950427
APPLICATION: US 1996-591632 19960220 (8) <--
WO 1994-US11907 19941019 19960220 PCT 371 date 19960220 PCT 102(e) date
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention describes synthetic human monoclonal antibodies that immunoreact with and neutralize **human immunodeficiency virus** (**HIV**). The synthetic monoclonal antibodies of this invention exhibit enhanced binding affinity and neutralization ability to **gp120**. Also disclosed are immunotherapeutic and diagnostic methods of using the monoclonal antibodies, as well as cell lines for producing the monoclonal antibodies.

CLM What is claimed is:
1. A human monoclonal antibody mutagenized to contain a complementary determining region that immunoreacts with and neutralizes **human immunodeficiency virus**-1 (**HIV**-1), wherein the monoclonal antibody reduces **HIV**-1 infectivity titer in an in vitro virus infectivity assay by 50% at a concentration of from 5 to 100 nanograms (ng) of antibody per milliliter (ml).

2. The human monoclonal antibody of claim 1 wherein said concentration is less than 20 ng/ml.

3. The human monoclonal antibody of claim 1 wherein said concentration is less than 10 ng/ml.

4. The human monoclonal antibody of claim 1 wherein said **HIV**-1 is a first **HIV**-1 strain and wherein said monoclonal antibody has the

capacity to reduce said **HIV**-1 infectivity titer of a second strain of **HIV**-1 by 50% at a concentration of less than 10 micrograms (ug) of antibody per milliliter (ml).

5. The human monoclonal antibody of claim 1 wherein said antibody is a Fab fragment.

6. The antibody of claim 1 wherein the complementary determining region is in a light chain immonoglobulin variable region.

7. The antibody of claim 1 wherein the complementary determining region is in a heavy chain immunoglobulin variable region.

8. The human monoclonal antibody of claim 7 wherein the heavy chain immunoglobulin variable region comprises an amino acid residue sequence having the sequence of SEQ ID NO 2, 3, 4 or 5.

9. The human monoclonal antibody of claim 1 that comprises an amino acid residue sequences in pairs of SEQ ID NOs 2:6, 3:6, 4:6 or 5:6.

10. The human monoclonal antibody of claim 1 wherein said monoclonal antibody immunoreacts with **HIV**-1 **gp120** with a dissociation constant (K_d) of 1×10^{-8} M or less.

11. The human monoclonal antibody of claim 7 wherein the heavy chain immunoglobulin variable region comprises an amino acid residue sequence having the sequence of SEQ ID NOs 1, 3, 54, 55, 56, 57, 58, 59, 89, 90, 91 or 92.

12. The human monoclonal antibody of claim 6 wherein the light chain immunoglobulin variable region comprises an amino acid residue sequence having the sequence of SEQ ID NOs 6, 69, 70, 73, 75, 76, 77, 79, 80, 82, 83, 84, 85, 86, 87 or 88.

13. The human monoclonal antibody of claim 10 wherein said dissociation constant is from 1×10^{-9} M to 1×10^{10} M.

14. The human monoclonal antibody of claim 1 that comprises at least one amino acid residue sequence in pairs of SEQ ID NOs 3:6, 3:69, 3:70, 3:73, 3:75, 3:76, 3:77, 3:79, 3:80, 3:82, 3:83, 3:84, 3:87, 54:6, 55:6, 56:6, 57:6, 58:6, 59:6, 90:88, 91:6, 91:88 or 92:88.

15. The human monoclonal antibody of claim 10 wherein said dissociation constant is from 1×10^{-10} M to 1×10^{11} M.

16. The human monoclonal antibody of claim 10 wherein said dissociation constant is from 1×10^{-11} M to 1×10^{-12} M.

17. A polynucleotide sequence encoding a heavy chain immunoglobulin variable region amino acid residue sequence of a mutagenized human monoclonal antibody that immunoreacts with **human immunodeficiency virus**-1 (**HIV**-1) glycoprotein **gp120** and neutralizes **HIV**-1, wherein the heavy chain immunoglobulin variable region comprises an amino acid residue sequence having the sequence of SEQ ID NOs 1, 2, 3, 4, 5, 54, 55, 56, 57, 58, 59, 89, 90, 91 or 92, and polynucleotide sequences complementary thereto.

18. A polynucleotide sequence encoding a light chain immunoglobulin variable region amino acid residue sequence of a mutagenized human monoclonal antibody that immunoreacts with **human immunodeficiency virus**-1 (**HIV**-1) glycoprotein **gp120** and neutralizes **HIV**-1, wherein the light chain immunoglobulin variable region comprises an amino acid residue sequence having the sequence of SEQ ID Nos 6, 69, 70, 73, 75, 76, 77, 79, 80, 82, 83, 84, 85, 86, 87 or 88, and polynucleotide sequences complementary thereto.

19. A polynucleotide sequence encoding a heavy and light chain immunoglobulin variable region amino acid residue sequence of a mutagenized human monoclonal antibody that immunoreacts with **human immunodeficiency virus**-1 (**HIV**-1) glycoprotein **gp120** and neutralizes **HIV**-1, wherein the heavy and light chain immunoglobulin variable regions comprise an amino acid residue sequence in pairs of SEQ ID NOs 2:6, 3:6, 4:6, 5:6, 3:69, 3:70, 3:73, 3:75, 3:76, 3:77, 3:79, 3:80, 3:82, 3:83, 3:84, 3:85, 3:86, 3:87, 54:6, 55:6, 56:6, 57:6, 58.6, 59:6, 89:6, 89:88, 90:86, 90:88, 91:6, 91:88 or 92:88, and polynucleotide sequences complementary thereto.

20. A host cell comprising the polynucleotide sequence of claims 17, 18 or 19.

21. A DNA expression vector comprising the polynucleotide sequence of claims 17, 18 or 19.

22. A method of detecting **human immunodeficiency virus** (**HIV**) comprising contacting a sample suspected of containing **HIV** with a diagnostically effective amount of the monoclonal antibody of claim 1 and determining whether the monoclonal antibody immunoreacts with the sample.

23. The method of claim 22, wherein the detecting is in vivo.

24. The method of claim 23, wherein the monoclonal antibody is detectably labelled with a label selected from the group consisting of a radioisotope and a **paramagnetic** label.

25. The method of claim 22, wherein the detecting is in vitro.

26. The method of claim 25, wherein the monoclonal antibody is detectably labelled with a label selected from the group consisting of a radioisotope, a fluorescent compound, a colloidal metal, a chemiluminescent compound, a bioluminescent compound, and an enzyme.

27. The method of claim 25, wherein the monoclonal antibody is bound to a solid phase.

28. A method for producing a mutagenized human anti-**HIV**-1 monoclonal antibody comprising the steps of: a) providing the genome of filamentous phage encoding a human monoclonal antibody having immunoglobulin heavy and light chain variable domains, said heavy chain variable domain present as a fusion polypeptide containing a filamentous phage membrane anchor domain, wherein said monoclonal antibody immunoreacts with **HIV**-1 glycoprotein **gp120**; b) mutating the immunoglobulin heavy chain variable domain-coding nucleotide sequence present in the provided genome to form a first library of mutagenized phage particles containing a mutated immunoglobulin heavy chain variable domain nucleotide sequence; c) contacting the library formed in step (b) with a **HIV**-1 glycoprotein **gp120** ligand under conditions sufficient for members of the library to bind to the ligand and form a first ligand-phage particle complex; d) isolating phage particles in said first complex away from non-bound library members to form a first ligand-enriched library comprising phage particles having binding specificity for said **HIV**-1 glycoprotein **gp120** ligand; e) providing the genome of filamentous phage from said first ligand-enriched library; f) mutating the immunoglobulin heavy chain variable domain-coding nucleotide sequence present in the provided genome to form a second library of mutagenized phage particles containing a mutated immunoglobulin heavy chain variable domain nucleotide sequence; g) contacting the library formed in step (f) with a **HIV**-1 glycoprotein **gp120** ligand under conditions sufficient for members of the library to bind to the ligand and form a second ligand-phage particle complex; and h) isolating phage particles in said second complex away from non-bound library members to form a second ligand-enriched library comprising phage particles having binding

specificity for said preselected **HIV**-1 ligand, thereby isolating a synthetic human monoclonal antibody immunoreactive with **HIV**-1.

29. The method of claim 28 wherein said mutating in steps (b) and (f) are directed to the same region of the immunoglobulin heavy chain variable domain.

30. The method of claim 28 wherein said mutating in steps (b) and (f) are directed to two different regions of the immunoglobulin heavy chain variable domain.

31. The method of claim 28 wherein said immunoglobulin heavy chain variable domain is a complementarity determining region (CDR) selected from the group consisting of CDR1, CDR2 and CDR3.

32. The method of claim 31 wherein said mutating in step (b) is directed to a first CDR and said mutating in step (f) is directed to a second CDR.

33. The method of claim 32 wherein said first and second CDR's are CDR1 and CDR3, respectively.

34. The method of claim 28 wherein said mutating of step (b) comprises inducing mutagenesis in a CDR of an immunoglobulin gene in said genome which comprises amplifying a portion of said CDR of the immunoglobulin gene by polymerase chain reaction (PCR) using a PCR primer oligonucleotide, said oligonucleotide having 5' and 3' termini and comprising: a) a nucleotide sequence at said 5' terminus capable of hybridizing to a framework region upstream of said CDR; b) a nucleotide sequence at said 3' terminus capable of hybridizing to a framework region downstream of said CDR; and c) a nucleotide sequence between said 5' and 3' termini according to the formula: $[NNS]_n$, wherein N is independently any nucleotide, S is G or C, and n is 3 to 24, said 3' and 5' terminal nucleotide sequences having a length of 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto.

35. The method of claim 34 wherein n is 5, said CDR is CDR1, and said upstream and downstream framework regions are FR1 and FR2, respectively.

36. The method of claim 28 wherein said mutating of step (f) comprises inducing mutagenesis in a CDR of an immunoglobulin gene in said genome which comprises amplifying a portion of said CDR of the immunoglobulin gene by polymerase chain reaction (PCR) using a PCR primer oligonucleotide, said oligonucleotide having 5' and 3' termini and comprising: a) a nucleotide sequence at said 5' terminus capable of hybridizing to the antisense (noncoding) framework region downstream of said CDR; b) a nucleotide sequence at said 3' terminus capable of hybridizing to the antisense (noncoding) framework region upstream of said CDR; and c) a nucleotide sequence between said 5' and 3' termini according to the formula: $[MNN]_n$, wherein N is independently any nucleotide, M is A or C, and n is 3 to 24, said 3' and 5' terminal nucleotide sequences having a length of 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto.

37. The method of claim 36 wherein n is 4, said CDR is CDR3, and said upstream and downstream framework regions are FR3 and FR4, respectively.

38. The method of claim 28 wherein said second ligand-enriched library comprises phage particles that contain synthetic antibody molecules that have the capacity to reduce **HIV**-1 infectivity titer in an in vitro virus infectivity assay by 50% at a concentration of less than 100 nanograms (ng) of antibody per milliliter (ml) of culture medium.

39. A synthetic monoclonal antibody produced by the method of claim 38.

40. An antibody produced by the process of claim 28.

L22 ANSWER 11 OF 19 USPATFULL on STN
2001:114495 REAGENT SYSTEM AND KIT FOR DETECTING **HIV** INFECTED CELLS.
KING, CHESTER F., FREDERICK, MD, United States
HALLOWITZ, ROBERT A., GAITHERSBURG, MD, United States
US 2001008760 A1 20010719
APPLICATION: US 1998-139663 A1 19980825 (9) <--
WO 1997-US18649 19971015 None PCT 102(e) date
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to blood collection and diagnostics. More particularly, the invention relates to blood collection and diagnostics utilizing techniques such as **magnetic** separation and photodetection. The present invention also relates to methods and an apparatus for detecting the presence of antigens displayed on the surface of cells. More preferably, the present invention relates to the detection of cells infected by **human immunodeficiency virus** (**HIV**) and related viruses. In accordance with the present invention, **HIV**-infected cells can be detected and separated from uninfected cells. In a preferred embodiment, separation is achieved by a **magnetic** field. By coating the infected cells with **magnetic** particles, transfer of the cells to a precise location is facilitated. A novel aspect of the present invention is a cartridge antigen test which allows for the collection and mixing of blood with reagents in one package, which can be viewed on a fluorescent microscope.

CLM What is claimed is:

1. A method of separating cells expressing a viral antigen, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the mixture, a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to the viral antigen on the cell surface; c) adding to the mixture resulting from b), a second binding partner specific for the first binding partner and attached to a **magnetic** bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and d) separating target cells containing the complex, whereby said separation is achieved by a **magnetic** field.

2. A method of claim 1, further comprising adding to the target cell a sample antibody specific for the viral antigen.

3. A method of claim 2, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample antibody

4. A method of claim 1, further comprising adding to the target cell a sample comprising an antibody specific for the viral antigen, whereby the amount of the second antibody is effective for interfering with the binding of the first binding partner to the viral antigen.

5. A method of claim 1, further comprising adding to the target cell a sample suspected of containing an antibody specific for the viral antigen.

6. A method of claim 5, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample.

7. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen.

8. A method of claim 6, wherein the second binding partner is an antibody specific for the first binding partner.

9. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen, which antibody is labeled with a detectable label.

10. A method of claim 9, wherein the second binding partner is an antibody specific for the detectable label.

11. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen, which antibody is labeled with a detectable label.

12. A method of claim 6, wherein the virus is **HIV**.

13. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen **gp120**, which antibody is labeled with a detectable label; and the second binding partner is an antibody specific for the detectable label.

14. A method of claim 6, wherein the target cell is a T-cell line.

15. A method of claim 6, wherein the sample is a body fluid or blood.

16. A method of claim 6, wherein measurement of the number of target cells separated in d) in the presence and absence of the sample is accomplished by flow cytometry.

17. A method of claim 12, wherein the first binding partner is a receptor for the viral antigen.

18. A method of claim 16, wherein the first binding partner is a receptor for the viral antigen and is labeled with a detectable label; and the second binding partner is an antibody specific for the detectable label.

19. A method of claim 6, wherein the bead diameter is about 50-120 nm.

20. A method of claim 6, wherein the cell is contacted by at least about 100-1000 beads.

21. A method of identifying an agent which interferes with viral infection of a cell, a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test sample containing an agent suspected with interfering with viral infection of the test cell; c) adding to the mixture of b), a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen on the cell surface; d) adding to the resultant mixture formed in c), a second binding partner specific for the first binding partner and to a **magnetic** bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; e) separating test cells containing said complex, whereby said separation is achieved by a **magnetic** field; and f) determining the number of cells infected with said virus in the presence and the absence of said test agent.

22. A **magnetic** bead having a surface coated by a cell-surface virus receptor for **HIV**.

23. A **magnetic** bead of claim 21, wherein the virus receptor is **CD4**.

24. A method of separating virus-infected cells from non-virus infected cells in a sample comprising, combining (a) a first antibody

recognizing a viral antigen on the surface of said cell and attached to a **magnetic** particle; (b) a second antibody recognizing said viral antigen on the surface of said cell and attached to a detectable label; and (c) a sample containing said virus-infected cells, to form a mixture; incubating said mixture under conditions effective for binding of said antibodies to said viral antigen to form a complex, said complex comprising said first and second antibody bound to said virus-infected cell, and moving said **magnetic** particle to a predetermined point on a reaction vessel holding said mixture, whereby said moving is accomplished by a **magnetic** field acting on said **magnetic** particle resulting in separating said virus-infected cells from non-virus infected cells, wherein said moving is accomplished without removing unbound antibody first and second antibody from said mixture.

25. A method of claim 24, further comprising detecting the label of said second antibody bound to said viral antigen on said virus-infected cell, wherein said first and second antibody recognize different epitopes of said viral antigen.

26. A method of separating cells infected with a virus, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus; b) fixing and permeabilizing said cells; c) adding to the fixed and permeabilized cells, a first binding partner specific for an antigen coded for by the virus, which viral antigen is ultimately expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to said viral antigen on the inside of said fixed and permeabilized cell; d) adding to the result of c), a second binding partner specific for the first binding partner and attached to a **magnetic** bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and e) separating target cells containing the complex, whereby said separation is achieved by a **magnetic** field.

27. A method of identifying an agent which interferes with viral infection of a cell, comprising: a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test agent suspected with interfering with viral infection of the test cell; c) fixing and permeabilizing said cells; d) adding a first binding partner specific for an antigen coded for by the virus, which viral antigen is expressed ultimately on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen when said viral antigen is expressed in the interior of said cell; e) adding to the resultant mixture formed in d), a second binding partner specific for the first binding partner and to a **magnetic** bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; f) separating test cells containing said complex, whereby said separation is achieved by a **magnetic** field; and g) determining whether the test sample changes the number of test cells containing the complex when compared to the process performed in the absence of said agent.

28. A method claim 27, where said test agent is added to cells prior to simultaneous to contacting cell with said test agent.

29. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen antibody attached to a detectable label, an effective amount of an antibody specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture, wherein said antibody specific-for said detectable label is attached to a **magnetic** particle;

b) incubating said mixture under conditions effective for binding of said anti-cell surface viral antibody to said cell-surface viral antigen, and, for binding of said antibody specific-for said detectable label to said detectable label attached to said anti-cell surface viral antibody, to form a complex, wherein said anti-viral antibody is bound to said cell-surface antigen displayed on a viral-infected cell; and c) separating said complex, comprising said cells expressing said cell-surface viral antigen and **magnetic** particles, by applying a **magnetic** field to said mixture, whereby said complex is retained by said **magnetic** field.

30. A method of claim 29, wherein viral-infected cells are infected with **HIV**.

31. A method of claim 29, wherein said cell-surface viral antigen is an envelope glycoprotein for **HIV**.

32. A method of claim 29, wherein the envelope glycoprotein is **gp120** or gp41.

33. A method of claim 29, wherein said anti-cell surface viral antibody is a polyclonal antibody specific for **HIV** envelope glycoprotein and said viral-infected cells are infected with **HIV**.

34. A method of claim 29, wherein said detectable label is FITC, TRITC, or R-phycoerthryin.

35. A method of claim 29, further comprising counting said magnetically-separated cells by flow cytometry.

36. A method of claim 29, wherein said **magnetic** particles are about 10-150 nm in diameter. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen antibody attached to a **magnetic** particle and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture; b) incubating said mixture under conditions effective for binding of said anti-cell surface viral antibody to said cell-surface viral antigen displayed on said viral-infected cells, to form a complex; and c) separating said complex comprising said cells expressing said cell-surface viral antigen and **magnetic** particles by applying a **magnetic** field to said mixture, whereby said complex is retained by said **magnetic** field.

L22 ANSWER 10 OF 19 USPATFULL on STN
2001:199904 METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD.
HALLOWITZ, ROBERT, GAITHERSBURG, MD, United States
SALAS, VIRGINIA, ALBUQUERQUE, NM, United States
US 2001039007 A1 20011108
APPLICATION: US 1999-296534 A1 19990422 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a new **HIV** status of a patient called "latent viral load." To measure the "latent viral load," in accordance with a preferred embodiment of the present invention, a population of sample cells is obtained from a desired source, such as an infected patient. The sample cell population is depleted of overtly infected cells and cells harboring active virus, to produce a subset of "resting cells" comprising uninfected and latently-infected cells. This subset is treated with an agent and/or condition that activates the latent virus in the host cell genome and results in a productive infection. The thus-produced infection reflects the "latent viral load" of the host because it reveals the presence of quiescent virus in cells. The latent viral load is useful in assessing a patient's disease status and the efficacy of highly active antiretroviral therapy and other treatment

protocols.

CLM What is claimed is:

1. A method of determining the latent viral load in a host infected with **HIV** comprising, treating resting lymphoid mononuclear cells obtained from the host with an effective amount of an agent capable of activating an **HIV** virus integrated into the genome of the cells; and detecting the expression of cell-surface **gp120** after the cells have been treated with the agent, wherein the presence or amount of cells expressing cell-surface **gp120** is a measure of latent viral load.

2. A method of claims 1, further comprising obtaining the resting lymphoid mononuclear cells by the steps of: a) obtaining a sample cell population; b) depleting the sample cell population of cells expressing cell-surface **gp120**; and c) depleting sample cell population of cells expressing HLA-DR.

3. A method of claim 2, wherein the sample cells are depleted of **gp120** expressing cells by the steps of: a) contacting sample cells with **gp120**-specific antibodies, each conjugated to a capture moiety, under conditions effective for the antibodies to attach to **gp120** on the surface of cells, thereby forming labeled-cells; b) contacting the labeled-cells with capture moiety-specific antibody under conditions effective for the capture moiety-specific antibody to attach to the labeled-cells, thereby forming a complex-labeled cells; and c) removing the complex-labeled cells, thereby depleting sample cells of **gp120+** cells.

4. A method of claim 3, wherein the capture moiety-specific antibody is conjugated to **magnetic** particles.

5. A method of claim 3, wherein the capture moiety is FITC and the capture moiety-specific antibody is FITC-specific antibody conjugated to a **magnetic** bead.

6. A method of claims 4, wherein the **magnetic** particles are 10-100 nm in diameter.

7. A method of claims 5, wherein the **magnetic** particles are 10-100 nm in diameter.

8. A method of claims 3, wherein the removing is accomplished by a **magnetic** field acting on the **magnetic** particles.

9. A method of claim 2, further comprising: separating **CD4+** cells from the sample.

10. A method of claim 2, further comprising: separating CD8+ cells from the sample.

11. A method of claim 2, wherein the depleting sample cell population of cells expressing HLA-DR is accomplished by flow cytometry cell sorting and said cells are labeled with a fluorochrome-labeled antibody specific-for HLA-DR.

12. A method of claim 1, wherein the tissue is lymphoid.

13. A method of claims 1, wherein the agent is phorbol ester or a cytokine.

14. A method of claim 1, wherein the measure of latent viral load is number of cells expressing **gp120** after treating the resting with an effective amount of an agent capable of activating an **HIV** virus integrated into the genome of the cells.

15. A method of claim 1, wherein the measure of latent viral load is compared to an established cell line harboring latent **HIV**-1.

16. A method of claim 15, wherein the cell line is OM-10.1, U1, or Jurkat cells.

17. A method of treating a viral infection comprising measuring the latent viral load in an **HIV**-infected patient; and determining whether to administer to the patient an agent capable of activating an **HIV** virus integrated into the genome of a cell by the value of the latent viral load.

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=> d his

     (FILE 'HOME' ENTERED AT 13:37:53 ON 17 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
                E HALLOWITZ R A/IN
L1            7 S E4 OR E5
                E KROWKA JOHN/IN
L2            1 S E3
                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
L7          182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8          182 S L7 AND ANTIBOD?
L9           16 S L8 AND (GP120/CLM OR GP160/CLM)
L10          20 S L7 AND AY<2000
L11          15 S L10 NOT L9
L12        2375 S L6 AND ANTIBOD?
L13        1113 S L12 AND (ANTIBOD?/CLM)
L14         211 S L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERA
L15          19 S L14 AND AY<2001

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                E HALLOWITZ R A/AU
L16           3 S E3
L17           1 S E2

     FILE 'BIOSIS' ENTERED AT 14:24:22 ON 17 JUN 2004
                E HALLOWITZ R A/AU
L18           8 S E3 OR E4

     FILE 'MEDLINE' ENTERED AT 14:25:54 ON 17 JUN 2004
                E KROWKA J/AU
L19           9 S E3

     FILE 'USPATFULL' ENTERED AT 14:27:23 ON 17 JUN 2004
L20         800 S L6 AND (PARAMAGNETIC OR MAGNETIC)
L21          39 S L20 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)
L22          19 S L21 AND AY<2001

=> file medline
COST IN U.S. DOLLARS                                 SINCE FILE      TOTAL
                                                          ENTRY    SESSION
FULL ESTIMATED COST                                       31.41     211.93

FILE 'MEDLINE' ENTERED AT 14:33:58 ON 17 JUN 2004

 FILE LAST UPDATED: 16 JUN 2004 (20040616/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.
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 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s (HIV or human immunodeficiency virus)
        137516 HIV
       8558187 HUMAN
        113476 IMMUNODEFICIENCY
        376149 VIRUS
         43060 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L23     142285 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l23 and (gp120 or gp160)
          5888 GP120
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=> s l25 and (FRET or RET or resonance energy transfer)
           909 FRET
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        251158 RESONANCE
        189710 ENERGY
        171435 TRANSFER
          2502 RESONANCE ENERGY TRANSFER
                 (RESONANCE(W)ENERGY(W)TRANSFER)
L26          5 L25 AND (FRET OR RET OR RESONANCE ENERGY TRANSFER)

=> d l26,ti,1-5

L26  ANSWER 1 OF 5     MEDLINE on STN
TI   The cell death-inducing ability of glycoprotein 120 from different HIV
     strains correlates with their ability to induce CD4 lateral association
     with CD95 on CD4+ T cells.

L26  ANSWER 2 OF 5     MEDLINE on STN
TI   Human immunodeficiency virus type 1 membrane fusion mediated by a
     laboratory-adapted strain and a primary isolate analyzed by resonance
     energy transfer.

L26  ANSWER 3 OF 5     MEDLINE on STN
TI   Cross-linking of CD4 in a TCR/CD3-juxtaposed inhibitory state: a pFRET
     study.

L26  ANSWER 4 OF 5     MEDLINE on STN
TI   Cytochalasin D modulates CD4 crosslinking sensitive mitogenic signal in
     T lymphocytes.

L26  ANSWER 5 OF 5     MEDLINE on STN
TI   CD4 changes conformation upon ligand binding.


=> d l26,cbib,ab,1-5

L26  ANSWER 1 OF 5     MEDLINE on STN
1999433493.    PubMed ID: 10505674.   The cell death-inducing ability of
     glycoprotein 120 from different HIV strains correlates with their
     ability to induce CD4 lateral association with CD95 on CD4+ T cells.
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Bottarel F; Feito M J; Bragardo M; Bonissoni S; Buonfiglio D; DeFranco S; Malavasi F; Bensi T; Ramenghi U; Dianzani U. (Department of Medical Sciences, A. Avogadro University of Eastern Piedmont at Novara, Italy.) AIDS research and human retroviruses, (1999 Sep 20) 15 (14) 1255-63. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB CD4 cross-linking by **HIV gp120** triggers **CD4**+ T cell death. Several authors have suggested that this effect is mediated by CD95, but this possibility is debated by other authors. In a previous work, we found by co-capping that **gp120**(451) and gp120MN, but not **gp120**(IIIB), induce lateral association of **CD4** with CD95 on the T cell surface. In this work, we used fluorescence **resonance energy transfer** to confirm that **CD4**/CD95 lateral association is induced by **gp120**(451), but not **gp120**(IIIB). Moreover, we found that **gp120** ability to induce the **CD4**/CD95 association correlates with ability to induce cell death, since **gp120**(451) and gp120MN induced higher levels of cell death than did **gp120**(IIIB) in PHA-derived **CD4**+ T cell lines. CD95 involvement in **gp120**-induced cell death was confirmed by showing that **gp120**(451) and gp120MN did not induce death in **CD4**+ T cells derived from patients with autoimmune/lymphoproliferative disease (ALD) and decreased CD95 function. Cell death induced by gp120MN was inhibited by a recombinant CD95/IgG.Fc molecule blocking the CD95/CD95L interaction. However, inhibition was late and only partial. These data suggest that the **gp120**-induced **CD4**/CD95 association exerts a dual effect: an early effect that is independent of CD95L and may be due to direct triggering of CD95 by **gp120**, and a late effect that may be due to sensitization of CD95 to triggering by CD95L. In line with the former effect, cell treatment with gp120MN activated caspase 3 in the presence of Fas/IgG.Fc, which shows that cell death induced by gp120MN independently of CD95L uses the same pathway as CD95.

L26 ANSWER 2 OF 5 MEDLINE on STN

96323171. PubMed ID: 8709277. **Human immunodeficiency virus** type 1 membrane fusion mediated by a laboratory-adapted strain and a primary isolate analyzed by **resonance energy transfer**. Litwin V; Nagashima K A; Ryder A M; Chang C H; Carver J M; Olson W C; Alizon M; Hasel K W; Maddon P J; Allaway G P. (Progenics Pharmaceuticals, Inc., Tarrytown, New York 10591, USA.) Journal of virology, (1996 Sep) 70 (9) 6437-41. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Previous studies of **human immunodeficiency virus** type 1 (**HIV**-1) envelope glycoprotein-mediated membrane fusion have focused on laboratory-adapted T-lymphotropic strains of the virus. The goal of this study was to characterize membrane fusion mediated by a primary **HIV**-1 isolate in comparison with a laboratory-adapted strain. To this end, a new fusion assay was developed on the basis of the principle of **resonance energy transfer**, using HeLa cells stably transfected with **gp120**/gp41 from the T-lymphotropic isolate **HIV**-1LA1 or the macrophage-tropic primary isolate **HIV**-1JR-FL. These cells fused with **CD4**+ target cell lines with a tropism mirroring that of infection by the two viruses. Of particular note, HeLa cells expressing **HIV**-1JR-FL **gp120**/gp41 fused only with PM1 cells, a clonal derivative of HUT 78, and not with other T-cell or macrophage cell lines. These results demonstrate that the envelope glycoproteins of these strains play a major role in mediating viral tropism. Despite significant differences exhibited by **HIV**-1JR-FL and **HIV**-1LAI in terms of tropism and sensitivity to neutralization by **CD4**-based proteins, the present study found that membrane fusion mediated by the envelope glycoproteins of these viruses had remarkably similar properties. In particular, the degree and kinetics of membrane fusion were similar, fusion occurred at neutral pH and was dependent on the presence of divalent cations. Inhibition of **HIV**-1JR-FL envelope glycoprotein-mediated membrane fusion by soluble **CD4** and **CD4**-IgG2 occurred at concentrations similar to those required to neutralize this virus. Interestingly, higher concentrations of these agents were required to inhibit **HIV**-1LAI envelope glycoprotein-mediated membrane fusion, in contrast to the greater sensitivity of **HIV**-1LAI

virions to neutralization by soluble **CD4** and **CD4**-IgG2. This finding suggests that the mechanisms of fusion inhibition and neutralization of **HIV**-1 are distinct.

L26 ANSWER 3 OF 5 MEDLINE on STN
95276119. PubMed ID: 7538802. Cross-linking of **CD4** in a TCR/CD3-juxtaposed inhibitory state: a pFRET study. Szabo G Jr; Weaver J L; Pine P S; Rao P E; Aszalos A. (Department of Biophysics, University Medical School of Debrecen, Hungary.) Biophysical journal, (1995 Mar) 68 (3) 1170-6. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB Instances when T cell activation via the T cell receptor/CD3 complex is suppressed by anti-**CD4** Abs are generally attributed either to the topological separation of **CD4**-p56lck from CD3, or their improper apposition. Photobleaching fluorescence **resonance energy transfer** measurements permitted direct analysis of these alternatives on human peripheral blood lymphocytes. Distinction between changes of relative antigen densities or positioning was made possible by simultaneously recording donor and acceptor fluorescence in the energy transfer experiment performed on homogeneous populations of flow-sorted cells. We show here that **CD4** stays in the molecular vicinity of CD3, while anti-CD3 stimulation is suppressed by anti-**CD4** or cross-linked **HIV gp120**. Our data suggest that cross-linking of **CD4** through particular epitopes is capable of inhibiting activation driven by Abs binding to specific sites on CD3 without major topological sequestration of the Ags, in such a way that additional positive signals will also be affected. Thus, these and other related cases of negative signaling via **CD4** may be interpreted in terms of functional uncoupling rather than a wide physical separation of **CD4** from the T cell receptor-CD3 complex.

L26 ANSWER 4 OF 5 MEDLINE on STN
94313697. PubMed ID: 7913666. Cytochalasin D modulates **CD4** crosslinking sensitive mitogenic signal in T lymphocytes. Aszalos A; Pine P S; Weaver J L; Rao P E. (Center for Drug Evaluations and Research, FDA, Washington, DC 20204.) Cellular immunology, (1994 Aug) 157 (1) 81-91. Journal code: 1246405. ISSN: 0008-8749. Pub. country: United States. Language: English.

AB It has previously been shown that crosslinking of the **CD4** molecule, either with anti-Leu3a mAb or with **gp120** (the **HIV** coat protein) plus anti-**gp120** mAb, suppresses activation induced by wt31, a TcR/CD3-specific mAb. This suppression was associated with hindrance of the necessary association of the p56lck kinase bearing **CD4** molecule with the TcR/CD3 complex. In this paper we demonstrate that this crosslinking-induced suppression can be bypassed by perturbing the microfilament system of **CD4**+ cells by pretreatment with 1 microM cytochalasin D. Using the fluorescence **resonance energy transfer** method, we have shown that the cytochalasin D-affected increase of mitogenesis is not due to changes in the TcR/CD3 to **CD4** distance. Likewise, other membrane biophysical parameters, membrane potential and lateral diffusion of surface receptors, cannot be associated with these cytochalasin D-affected mitogenic changes. Cytochalasin D treatment elevates intracellular Ca2+ levels induced by wt31 mAb plus crosslinking and generates a TcR/CD3-dependent signal which is cyclosporin sensitive.

L26 ANSWER 5 OF 5 MEDLINE on STN
93056534. PubMed ID: 1431129. **CD4** changes conformation upon ligand binding. Szabo G Jr; Pine P S; Weaver J L; Rao P E; Aszalos A. (Center for Drug Evaluation and Research, FDA, Washington, DC 20204.) Journal of immunology (Baltimore, Md. : 1950), (1992 Dec 1) 149 (11) 3596-604. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Aurintricarboxylic acid (ATA) has been shown to block the binding site for both **HIV gp120** and mAb anti-Leu 3a on **CD4**. We have unexpectedly found that brief treatment with > or = 1 micrograms/ml ATA rapidly disengages another mAb, OKT4E, after it has been bound to **CD4** on human PBL. OKT4E is specific for a discontinuous epitope overlapping the MHC class II-binding region in the N-terminal **CD4** domain. Interestingly,

among 10 other mAb tested, only anti-Leu 8, specific for a leukocyte homing receptor is also quickly released from the cells by ATA treatment. Disengagement of the OKT4E mAb is also seen on a **CD4**-positive cell line (HPB-ALL) and with recombinant soluble **CD4** (sCD4) bound to immobilized OKT4E. In all of these cases, disengagement is prevented if OKT4E is cross-linked, or the Leu 3a site is blocked by the mAb, but not by **gp120**. Photobleaching fluorescence **resonance energy transfer** (pFRET) measurements suggest that OKT4E is released as an indirect consequence of ATA-evoked conformational changes of **CD4**. Similar changes were detected as a result of **gp120** binding to PBL. These data raise the possibility of a novel type of immunomodulation: induced disengagement of a bound ligand from its Ag.

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=> d his

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L1            7 S E4 OR E5
                E KROWKA JOHN/IN
L2            1 S E3
                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
L7          182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8          182 S L7 AND ANTIBOD?
L9           16 S L8 AND (GP120/CLM OR GP160/CLM)
L10          20 S L7 AND AY<2000
L11          15 S L10 NOT L9
L12        2375 S L6 AND ANTIBOD?
L13        1113 S L12 AND (ANTIBOD?/CLM)
L14         211 S L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERA
L15          19 S L14 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 14:21:56 ON 17 JUN 2004
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L17           1 S E2

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                E HALLOWITZ R A/AU
L18           8 S E3 OR E4

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                E KROWKA J/AU
L19           9 S E3

     FILE 'USPATFULL' ENTERED AT 14:27:23 ON 17 JUN 2004
L20         800 S L6 AND (PARAMAGNETIC OR MAGNETIC)
L21          39 S L20 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)
L22          19 S L21 AND AY<2001

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L23      142285 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L24        6469 S L23 AND (GP120 OR GP160)
L25        2828 S L24 AND (CD4?)
L26           5 S L25 AND (FRET OR RET OR RESONANCE ENERGY TRANSFER)

=> s l23 and (infected cells or infectivity status)
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       1623180 CELLS
         19604 INFECTED CELLS
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        317799 STATUS
             8 INFECTIVITY STATUS
                 (INFECTIVITY(W)STATUS)
L27       2811 L23 AND (INFECTED CELLS OR INFECTIVITY STATUS)

=> s l23 and (infectivity status)
         11750 INFECTIVITY
        317799 STATUS
             8 INFECTIVITY STATUS
                 (INFECTIVITY(W)STATUS)
L28          0 L23 AND (INFECTIVITY STATUS)

=> s l27 and (gp120 or gp160)
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       1929073 DIAGNOS?
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       1783989 CELL
        613159 NUMBER
          9846 CELL NUMBER
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L31          4 L30 AND (QUANTITATIVE? OR CELL NUMBER)

=> d l31,cbib,1-4
```

L31 ANSWER 1 OF 4 MEDLINE on STN
1998252382. PubMed ID: 9591708. Phase I study of a human monoclonal antibody directed against the CD4-binding site of **HIV** type 1 glycoprotein 120. Cavacini L A; Samore M H; Gambertoglio J; Jackson B; Duval M; Wisnewski A; Hammer S; Koziel C; Trapnell C; Posner M R. (Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215, USA.) AIDS research and human retroviruses, (1998 May 1) 14 (7) 545-50. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

L31 ANSWER 2 OF 4 MEDLINE on STN
92075335. PubMed ID: 1742075. Initial stages of **HIV**-1 envelope glycoprotein-mediated cell fusion monitored by a new assay based on redistribution of fluorescent dyes. Dimitrov D S; Golding H; Blumenthal R. (Section on Membrane Structure and Function, NCI, NIH, Bethesda, MD 20892.) AIDS research and human retroviruses, (1991 Oct) 7 (10) 799-805. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

L31 ANSWER 3 OF 4 MEDLINE on STN
91291369. PubMed ID: 2064772. Use of a focal infectivity assay for testing susceptibility of **HIV** to antiviral agents. Pincus S H; Wehrly K; Chesebro B. (NIAID Rocky Mountain Laboratories.) BioTechniques, (1991 Mar) 10 (3) 336-42. Journal code: 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

L31 ANSWER 4 OF 4 MEDLINE on STN
88333153. PubMed ID: 3047430. Development of a sensitive **quantitative** focal assay for **human immunodeficiency virus** infectivity. Chesebro B; Wehrly K. (Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840.) Journal of virology, (1988 Oct) 62 (10)

3779-88. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

=> d l31,cbib,ab,1-4

L31 ANSWER 1 OF 4 MEDLINE on STN
1998252382. PubMed ID: 9591708. Phase I study of a human monoclonal antibody directed against the CD4-binding site of **HIV** type 1 glycoprotein 120. Cavacini L A; Samore M H; Gambertoglio J; Jackson B; Duval M; Wisnewski A; Hammer S; Koziel C; Trapnell C; Posner M R. (Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215, USA.) AIDS research and human retroviruses, (1998 May 1) 14 (7) 545-50. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB A phase I dose escalation study was conducted with the human monoclonal anti-**gp120** antibody F105, to evaluate the safety, pharmacokinetics, and functional activity of F105 in **HIV**-1-infected individuals. F105 is an IgG1(kappa) antibody reactive with a discontinuous epitope that overlaps the CD4-binding site of **gp120**. F105 neutralizes laboratory strains of **HIV**-1 and some primary isolates, and synergizes with other antibodies in neutralizing an expanded spectrum of isolates. Four patients each with CD4 counts between 200 and 500/mm3 received a single dose of F105 at 100 or 500 mg/m2, intravenously. Sustained levels of F105 were obtained in plasma, and there was no evidence of an immune response to F105 as determined by a double-antigen immunoassay. No patient experienced any toxicity. Infused antibody retained full functional activity as **detected** by the ability of sera to block the binding of labeled F105 to **HIV**-1-**infected cells**. Of note, all patients had preexisting antibody to the **gp120** CD4-binding site. The ability to culture virus by **quantitative** microculture remained unchanged by this single dose of antibody. Thus, it can be concluded that F105 is safe and nontoxic as a single injection at the doses tested. Furthermore, the antibody retains full **gp120**-binding activity. In these patients, with preexisting CD4-binding site antibody, there is no evidence of anti-**HIV**-1 activity following a single antibody infusion.

L31 ANSWER 2 OF 4 MEDLINE on STN
92075335. PubMed ID: 1742075. Initial stages of **HIV**-1 envelope glycoprotein-mediated cell fusion monitored by a new assay based on redistribution of fluorescent dyes. Dimitrov D S; Golding H; Blumenthal R. (Section on Membrane Structure and Function, NCI, NIH, Bethesda, MD 20892.) AIDS research and human retroviruses, (1991 Oct) 7 (10) 799-805. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Membrane fusion is an essential step in the infection of permissive cells with **human immunodeficiency virus** (**HIV**). **Infected cells** frequently fuse with each other, and then progress to form multinucleated giant cells (syncytia). To gain insight into mechanisms of **HIV** env-mediated membrane fusion, we developed a new assay for studying the initial events. The assay is based on the redistribution of fluorescent markers between membranes and cytoplasm of adjacent cells examined by means of fluorescence video microscopy. Membrane fusion between **HIV**-1 envelope glycoprotein (**gp120**/41) expressing effector cells and CD4+ target cells was observed 90 min after the association of cells, whereas the first syncytia only became apparent after 5 h. Moreover, membrane fusion events were observed under conditions where no syncytia were **detected**, for example, when the effector:target cell ratio was greater than 100:1, or less than 1:100. A significant number of cells with fused membranes were not involved in the syncytia. In order to determine whether **quantitative** differences in receptor expression might influence the extent of membrane fusion, we used laboratory-selected variants of CEM cells that differ in their expression of CD4. We found that CD4 is required on the target membrane for **HIV** env-mediated membrane fusion, but its extent is only partially dependent on CD4 surface concentration. The ability of those CEM variants to take part in **HIV** env-mediated

membrane fusion did not correlate with their capacity to form syncytia. These findings indicate that additional steps are needed to form syncytia after membrane fusion.

L31 ANSWER 3 OF 4 MEDLINE on STN
91291369. PubMed ID: 2064772. Use of a focal infectivity assay for testing susceptibility of **HIV** to antiviral agents. Pincus S H; Wehrly K; Chesebro B. (NIAID Rocky Mountain Laboratories.) BioTechniques, (1991 Mar) 10 (3) 336-42. Journal code: 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB A highly sensitive and **quantitative** focal immunoassay has been developed for **detecting** the **human immunodeficiency virus** (**HIV**). The assay can be used to measure cell-free virus or the production of **HIV** by virus-**infected cells**. Both laboratory-adapted strains of **HIV** and patient isolates can be studied with this assay. In this communication, we demonstrate the utility of this assay for measuring the effects of anti-**HIV** agents on viral isolates. We show that the anti-viral effects of such diverse agents as azidothymidine, interferon-alpha, immunotoxins, soluble CD4 and antibody can be accurately quantified. This assay may be used in the discovery and evaluation of new anti-**HIV** therapies or may be adapted for use in testing the sensitivity of patient isolates to standard therapeutic agents.

L31 ANSWER 4 OF 4 MEDLINE on STN
88333153. PubMed ID: 3047430. Development of a sensitive **quantitative** focal assay for **human immunodeficiency virus** infectivity. Chesebro B; Wehrly K. (Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840.) Journal of virology, (1988 Oct) 62 (10) 3779-88. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Accurate and sensitive quantitation of infectious **human immunodeficiency virus** (**HIV**) has been difficult to achieve. In this report, a **quantitative** focal immunoassay (FIA) for **HIV** was developed using human HeLa cells rendered susceptible to **HIV** infection by introduction of the CD4 gene via a retrovirus vector. **Infected cells** were identified by using human anti-**HIV** antibodies or mouse monoclonal antibodies specific for **HIV** together with secondary fluorescein- or peroxidase-conjugated antibody specific for mouse or human immunoglobulins. The assay identified cells infected with either wild-type or culture-adapted **HIV** isolates and was capable of **detecting** 1 positive cell in 10(6) cells. The FIA was also effective at **detecting** cell-free **HIV**, and in contrast to assays using A3.01, CEM, and other human leukemia cells, the FIA **detected** most wild-type **HIV** isolates. **HIV** neutralization could be determined by using the FIA, and two monoclonal antibodies reactive with **HIV gp120** were found to neutralize only the LAV-IIIB strain of **HIV**. These monoclonal antibodies, as well as antibodies in serum samples from patients with acquired immune deficiency syndrome, were able to inhibit the spread of **HIV** infection in human lymphocyte suspension cultures but not in CD4-positive HeLa cells growing attached to plastic dishes.

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L2            1 S E3
                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
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L6          2490 S L5 AND (CD4?)
L7           182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8           182 S L7 AND ANTIBOD?
L9            16 S L8 AND (GP120/CLM OR GP160/CLM)
L10           20 S L7 AND AY<2000
L11           15 S L10 NOT L9
L12         2375 S L6 AND ANTIBOD?
L13         1113 S L12 AND (ANTIBOD?/CLM)
L14          211 S L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERA
L15           19 S L14 AND AY<2001

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L18            8 S E3 OR E4

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L21           39 S L20 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)
L22           19 S L21 AND AY<2001

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L23       142285 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
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L25         2828 S L24 AND (CD4?)
L26            5 S L25 AND (FRET OR RET OR RESONANCE ENERGY TRANSFER)
L27         2811 S L23 AND (INFECTED CELLS OR INFECTIVITY STATUS)
L28            0 S L23 AND (INFECTIVITY STATUS)
L29          445 S L27 AND (GP120 OR GP160)
L30           85 S L29 AND (DETECT? OR DIAGNOS?)
L31            4 S L30 AND (QUANTITATIVE? OR CELL NUMBER)

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=> s l32 not l31
L33            5 L32 NOT L31

=> d l33,cbib,ab,1-5
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L33 ANSWER 1 OF 5 MEDLINE on STN
1999297581. PubMed ID: 10371171. The implication of the chemokine receptor CXCR4 in **HIV**-1 envelope protein-induced apoptosis is independent of the G protein-mediated signalling. Blanco J; Jacotot E; Cabrera C; Cardona A; Clotet B; De Clercq E; Este J A. (Institut de Recerca de la SIDA-Caixa, Laboratori de Retrovirologia, Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain.) AIDS (London, England), (1999 May 28) 13 (8) 909-17. Journal code: 8710219. ISSN: 0269-9370. Pub. country: ENGLAND: United Kingdom. Language: English.

AB OBJECTIVE: The envelope glycoprotein complex (**gp120**/gp41)n of **HIV**-1 is one of the viral products responsible for increased apoptosis in **HIV** infection. Here the role of the chemokine receptor CXCR4 in **HIV**-1 envelope protein-induced apoptosis was investigated. METHODS: Apoptosis occurring in cocultures of chronically **HIV**-1 IIIB-**infected cells** with CD4 target cells expressing the CXCR4 receptor was **quantified** by terminal deoxinucleotidyl transferase dUTP nick end labeling (TUNEL) or propidium iodide staining followed by fluorescent antibody cell sorting,

which allows the evaluation of single-cell killing. Moreover global (single cell- and syncytium-associated) apoptosis was **quantified** by a new radioactive TUNEL-derived assay. RESULTS: By using these different techniques it was shown that single and syncytium-forming CD4 T cells die by apoptosis upon contact with envelope protein expressing cells independently of viral replication. Moreover, both the CXCR4 agonist SDF-1alpha, and the antagonist AMD3100, showed inhibitory effects on **HIV**-1 envelope protein-induced apoptosis in the CD4 T-cell subset of peripheral blood mononuclear cells and CD4 cell lines. CXCR4 signalling-induced by **HIV**-1 envelope proteins in CD4 T cells was not **detected**. Furthermore, it was shown that envelope protein-induced apoptosis can occur after treating target cells with the Gi-protein inhibitor pertussis toxin. CONCLUSIONS: Evidence is provided for a role of CXCR4 in the mechanisms of **HIV** envelope protein-induced pathogenesis, contributing to selective CD4 cell killing. The results suggest that CXCR4 is involved in **HIV**-1-induced apoptosis; however, this role does not appear to involve G-protein-mediated CXCR4 signalling.

L33 ANSWER 2 OF 5 MEDLINE on STN
96038350. PubMed ID: 8530563. **Detection** of **HIV**-1 infection in vitro using NASBA: an isothermal RNA amplification technique. Romano J W; Shurtliff R N; Sarngadharan M G; Pal R. (Advanced BioSciences Laboratories Inc., Kensington, MD 20895, USA.) Journal of virological methods, (1995 Aug) 54 (2-3) 109-19. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB Establishment of a sensitive infection assay for **HIV**-1 is essential for successful screening of antiviral agents and neutralizing antibodies. In this report, an infection assay is described which measures the expression of viral genomic RNA and spliced mRNA intermediates in **infected cells** by an amplification-based technique called NASBA. The extreme sensitivity of this method permits the **detection** of viral RNA in peripheral blood mononuclear cells (PBMC) within 48 h of infection by a low dose of virus. Similarly, spliced **HIV**-1 mRNA could be **detected** within 24 h of infection of CEM cells by **HIV**-1IIIB. This NASBA-based infection assay was shown to titer the neutralization of the **HIV**-1IIIB isolate by serum from an infected human and by a monoclonal antibody to **gp120**. Furthermore, the inhibitory effects of azidothymidine (AZT) and soluble CD4 on **HIV**-1IIIB infection were **quantitated** by this assay. The early **detection** of virus by NASBA minimizes the contribution of secondary infection, thereby permitting more accurate evaluation of antiviral agents and neutralizing antibodies. This assay may be useful for the study of infection of phenotypically distinct **HIV**-1 isolates, which differ in terms of their replication kinetics.

L33 ANSWER 3 OF 5 MEDLINE on STN
94092368. PubMed ID: 8267903. Complement activation upon binding of mannan-binding protein to **HIV** envelope glycoproteins. Haurum J S; Thiel S; Jones I M; Fischer P B; Laursen S B; Jensenius J C. (Department of Immunology, University of Aarhus, Denmark.) AIDS (London, England), (1993 Oct) 7 (10) 1307-13. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB OBJECTIVE: Retroviruses can activate the complement system in the absence of antibodies, and the purpose of this study was to examine whether the serum collection, mannan-binding protein (MBP), could mediate such complement activation. DESIGN: Virus envelope proteins **gp120** and gp110 from **HIV**-1 and **HIV**-2 were incubated in microtitre wells coated with anti-**gp120** or anti-gp110 antibodies. After further incubation with serum, complement activation was measured as deposition of complement factor C4 and C3 onto the wells. Deposited C4 and C3 were **detected** with enzyme-labelled antibodies. Normal human serum depleted of endogenous lectins by affinity chromatography was used as the complement source. Serum from C1q-deficient patients was used in some experiments. Complement activation was then assessed with and without prior addition of MBP to the wells. Complement activation was also correlated with the **quantity** of endogenous MBP in a number of normal sera. RESULTS: Complement activation by **HIV** envelope glycoproteins was found to be

mediated by the binding of MBP to carbohydrates on natural envelope protein produced in virus-**infected cells**, as well as on glycosylated recombinant envelope proteins produced in insect cells. Non-glycosylated recombinant envelope proteins produced in Escherichia coli did not induce this type of complement activation. CONCLUSIONS: Activation of the classical complement pathway by retrovirus envelope proteins can be initiated by the binding of MBP to carbohydrate side chains of envelope glycoproteins.

L33 ANSWER 4 OF 5 MEDLINE on STN
94047336. PubMed ID: 8230445. Incorporation of Vpr into **human immunodeficiency virus** type 1 virions: requirement for the p6 region of gag and mutational analysis. Paxton W; Connor R I; Landau N R. (Aaron Diamond AIDS Research Center, New York, New York.) Journal of virology, (1993 Dec) 67 (12) 7229-37. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The product of the vpr open reading frame of **human immunodeficiency virus** type 1 (**HIV**-1) is a 15-kDa, arginine-rich protein that is present in virions in molar **quantities** equivalent to that of Gag. We report here the results of our investigations into the mechanism by which Vpr is incorporated into virions during assembly in **infected cells**. For these studies we used an expression vector encoding a Vpr molecule fused at its amino terminus to a nine-amino-acid peptide from influenza virus hemagglutinin. The tagged Vpr expression vector and a vpr mutant **HIV**-1 provirus were used to cotransfect COS cells, and the resulting virions were tested for the presence of the tagged protein on immunoblots probed with monoclonal antibody against the hemagglutinin peptide. The COS-produced virions were found to contain readily **detectable** amounts of tagged Vpr and smaller amounts of a putative tagged Vpr dimer. Infectivity of the particles was not altered by incorporation of tagged Vpr. Our results using this system in combination with mutant **HIV**-1 proviruses suggested that incorporation of Vpr into virions requires the carboxy-terminal Gag protein of **HIV**-1 (p6) but not **gp160**, Pol, or genomic viral RNA. In addition, analysis of mutated, tagged Vpr molecules suggested that amino acids near the carboxy terminus (amino acids 84 to 94) are required for incorporation of Vpr into **HIV**-1 virions. The single cysteine residue near the carboxy terminus was required for production of a stable protein. Arginine residues tested were not important for incorporation or stability of tagged Vpr. These results suggested a novel strategy for blocking **HIV**-1 replication.

L33 ANSWER 5 OF 5 MEDLINE on STN
93112220. PubMed ID: 1282012. Antibody-dependent cellular cytotoxicity (ADCC) is directed against immunodominant epitopes of the envelope proteins of **human immunodeficiency virus** 1 (**HIV**-1). Ziegner U H; Frank I; Bernatowicz A; Starr S E; Streckert H J. (Division of Infectious Diseases and Immunology, Children's Hospital of Philadelphia, Pennsylvania.) Viral immunology, (1992 Winter) 5 (4) 273-81. Journal code: 8801552. ISSN: 0882-8245. Pub. country: United States. Language: English.

AB In this study, epitopes of **HIV** envelope proteins that are involved in ADCC were identified. Peripheral blood mononuclear cells (PBMC) were obtained from adults with asymptomatic **HIV** infection or early symptoms of AIDS. These PBMC, which were reported to be "armed" in vivo with **HIV**-specific antibodies, were used as effector cells in 51Cr release assays. Target cells consisted of CD4 lymphocytes from healthy seronegative donors, coated with the IIIB strain of **HIV**-1 or with one of seven synthetic peptides. Cytotoxicity was **detected** against CD4 lymphocytes coated with **HIV**-1 IIIB or with the peptides env aa 507-518, corresponding to the carboxy-terminus of **gp120**, and env aa 597-611, corresponding to the region of the cysteine loop of gp41. The magnitude of target cell lysis was directly related to the **quantity** of peptide used. In contrast, target cells coated with the peptide gag aa 129-135, corresponding to the p17/p24 cleavage region of the gag precursor, were not killed. The same immunodominant regions which were involved in ADCC were recognized in enzyme-linked immunoabsorbent assays (ELISA) by the

majority of 107 sera from **HIV**-infected adults. We conclude that the immunodominant epitopes located at the carboxy-terminus of **gp120** and the cysteine loop of gp41 serve as recognition structure for antibodies, capable of mediating ADCC against **HIV-infected cells.**

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                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
L7          182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
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L9           16 S L8 AND (GP120/CLM OR GP160/CLM)
L10          20 S L7 AND AY<2000
L11          15 S L10 NOT L9
L12        2375 S L6 AND ANTIBOD?
L13        1113 S L12 AND (ANTIBOD?/CLM)
L14         211 S L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERA
L15          19 S L14 AND AY<2001

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                E HALLOWITZ R A/AU
L16           3 S E3
L17           1 S E2

     FILE 'BIOSIS' ENTERED AT 14:24:22 ON 17 JUN 2004
                E HALLOWITZ R A/AU
L18           8 S E3 OR E4

     FILE 'MEDLINE' ENTERED AT 14:25:54 ON 17 JUN 2004
                E KROWKA J/AU
L19           9 S E3

     FILE 'USPATFULL' ENTERED AT 14:27:23 ON 17 JUN 2004
L20         800 S L6 AND (PARAMAGNETIC OR MAGNETIC)
L21          39 S L20 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)
L22          19 S L21 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 14:33:58 ON 17 JUN 2004
L23      142285 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L24        6469 S L23 AND (GP120 OR GP160)
L25        2828 S L24 AND (CD4?)
L26           5 S L25 AND (FRET OR RET OR RESONANCE ENERGY TRANSFER)
L27        2811 S L23 AND (INFECTED CELLS OR INFECTIVITY STATUS)
L28           0 S L23 AND (INFECTIVITY STATUS)
L29         445 S L27 AND (GP120 OR GP160)
L30          85 S L29 AND (DETECT? OR DIAGNOS?)
L31           4 S L30 AND (QUANTITATIVE? OR CELL NUMBER)
L32           9 S L30 AND QUANTI?
L33           5 S L32 NOT L31

=> s l24 and (measur? or determin? or detect?)
       1203735 MEASUR?
       1338380 DETERMIN?
        830706 DETECT?
L34        2391 L24 AND (MEASUR? OR DETERMIN? OR DETECT?)
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=> s l24 and (FACS or fluorescent activated cell sort?)
          4298 FACS
        160102 FLUORESCENT
        210723 ACTIVATED
       1783989 CELL
         27720 SORT?
           213 FLUORESCENT ACTIVATED CELL SORT?
                 (FLUORESCENT(W)ACTIVATED(W)CELL(W)SORT?)
L35         16 L24 AND (FACS OR FLUORESCENT ACTIVATED CELL SORT?)

=> d l35,cbib,ab,1-16
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L35 ANSWER 1 OF 16 MEDLINE on STN

2004214572. PubMed ID: 15075509. Association of strong virus-specific CD4 T cell responses with efficient natural control of primary **HIV**-1 infection. Gloster Simone E; Newton Philippa; Cornforth David; Lifson Jeffrey D; Williams Ian; Shaw George M; Borrow Persephone. (Edward Jenner Institute for Vaccine Research, Compton, UK.) AIDS (London, England), (2004 Mar 26) 18 (5) 749-55. Journal code: 8710219. ISSN: 0269-9370. Pub. country: England: United Kingdom. Language: English.

AB OBJECTIVE: To investigate whether there are differences in the virus-specific CD4 T cell response during primary **HIV**-1 infection in patients who naturally (without antiretroviral intervention) control viral replication with differing efficiencies. METHODS: CD4 T cell responses to recombinant **HIV** proteins (Gag p24 and p55 and Env **gp160**) and an inactivated **HIV**-1 preparation were analysed using interferon-gamma ELISPOT assays (with CD8-depleted peripheral blood mononuclear cells) and by intracellular interferon-gamma staining and **fluorescent-activated cell sorting**. RESULTS: Strong **HIV**-specific CD4 T cell responses were detected from the earliest time-points analysed in primary infection in patients who naturally established low persisting viral loads. By contrast, **HIV**-specific CD4 T cell responses were weaker (at or just below the limit of detection in our assays) at similar time-points in patients who went on to establish high persisting viral loads. Statistical analysis revealed a highly significant difference (P < 0.001) between the magnitudes of the Gag p24-specific response at the earliest time-point analysed in primary infection in the two sets of patients. CONCLUSIONS: Strong **HIV**-specific CD4 T cell responses are associated with efficient natural control of primary **HIV**-1 infection.

L35 ANSWER 2 OF 16 MEDLINE on STN

2003235906. PubMed ID: 12759492. Complement-mediated enhancement of **HIV**-1 neutralisation by anti-HLA antibodies derived from polytransfused patients. Wilfingseder Doris; Spruth Martin; Ammann Christoph G; Dopper Susanne; Speth Cornelia; Dierich Manfred P; Stoiber Heribert. (Institute of Hygiene and Social Medicine, Innsbruck, Austria.) International archives of allergy and immunology, (2003 May) 131 (1) 62-72. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB We have recently shown that 'alloimmune sera' derived from polytransfused patients (PTP sera) are able to recognise and neutralise **HIV** in vitro. In this study we try to identify the protein(s), which are recognised by the PTP sera and elucidate mechanisms responsible for the neutralising capacity of these sera. The PTP sera allowed immunoprecipitation (IP) of HLA class II molecules on **HIV**-infected cells. To detect a potential cross-reactivity of alloreactive antibodies (Ab) with the **HIV** envelope protein **gp160** or its subunits **gp120**/gp41 and HLA proteins, ELISA and **FACS** analyses were performed. The lack of reactivity of the PTP sera against rsgp160 in ELISA or **FACS** analysis indicated that recognition of cells was independent of **HIV** infection. To clarify whether interaction of the PTP sera with target cells has any effect on the infection process, virus neutralisation assays were performed. Inhibition of **HIV** infection was observed only when virus was pre-incubated with the PTP sera. Complement enhanced neutralisation of **HIV**-1 significantly. This enhancement was not due to complement-mediated lysis, because

pre-incubation of the target cells with PTP sera did not inhibit **HIV** replication. Therefore, the neutralising effect of the Ab was due to blocking of the viral attachment/fusion process and not to negative signalling after infection. Since steric hindrance is possible only when HLA and **gp120**/gp41 are in close vicinity, isolation of rafts and IP assays were performed. These experiments revealed that **gp120** and MHC class II molecules are indeed co-localised. The close physical association of **gp120**/gp41 and HLA strongly supports a mechanism for neutralisation of **HIV** by anti-HLA-Ab based on steric hindrance.

L35 ANSWER 3 OF 16 MEDLINE on STN
2000456121. PubMed ID: 10835604. Paramagnetic proteoliposomes containing a pure, native, and oriented seven-transmembrane segment protein, CCR5. Mirzabekov T; Kontos H; Farzan M; Marasco W; Sodroski J. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115, USA.) Nature biotechnology, (2000 Jun) 18 (6) 649-54. Journal code: 9604648. ISSN: 1087-0156. Pub. country: United States. Language: English.

AB Seven-transmembrane segment, G protein-coupled receptors play central roles in a wide range of biological processes, but their characterization has been hindered by the difficulty of obtaining homogeneous preparations of native protein. We have created paramagnetic proteoliposomes containing pure and oriented CCR5, a seven-transmembrane segment protein that serves as the principal coreceptor for **human immunodeficiency virus** (**HIV**-1). The CCR5 proteoliposomes bind the **HIV**-1 **gp120** envelope glycoprotein and conformation-dependent antibodies against CCR5. The binding of **gp120** was enhanced by a soluble form of the other **HIV**-1 receptor, CD4, but did not require additional cellular proteins. Paramagnetic proteoliposomes are uniform in size, stable in a broad range of salt concentrations and pH, and can be used in **FACS** and competition assays typically applied to cells. Integral membrane proteins can be inserted in either orientation into the liposomal membrane. The magnetic properties of these proteoliposomes facilitate rapid buffer exchange useful in multiple applications. As an example, the CCR5-proteoliposomes were used to select CCR5-specific antibodies from a recombinant phage display library. Thus, paramagnetic proteoliposomes should be useful tools in the analysis of membrane protein interactions with extracellular and intracellular ligands, particularly in establishing screens for inhibitors.

L35 ANSWER 4 OF 16 MEDLINE on STN
2000429918. PubMed ID: 10933700. Characterization and epitope mapping of neutralizing monoclonal antibodies produced by immunization with oligomeric simian immunodeficiency virus envelope protein. Edinger A L; Ahuja M; Sung T; Baxter K C; Haggarty B; Doms R W; Hoxie J A. (Department of Pathology and Laboratory Medicine, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.) Journal of virology, (2000 Sep) 74 (17) 7922-35. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB In an attempt to generate broadly cross-reactive, neutralizing monoclonal antibodies (MAbs) to simian immunodeficiency virus (SIV), we compared two immunization protocols using different preparations of oligomeric SIV envelope (Env) glycoproteins. In the first protocol, mice were immunized with soluble gp140 (sgp140) from CP-MAC, a laboratory-adapted variant of SIVmacBK28. Hybridomas were screened by enzyme-linked immunosorbent assay, and a panel of 65 MAbs that recognized epitopes throughout the Env protein was generated. In general, these MAbs detected Env by Western blotting, were at least weakly positive in fluorescence-activated cell sorting (**FACS**) analysis of Env-expressing cells, and preferentially recognized monomeric Env protein. A subset of these antibodies directed toward the V1/V2 loop, the V3 loop, or nonlinear epitopes were capable of neutralizing CP-MAC, a closely related isolate (SIVmac1A11), and/or two more divergent strains (SIVsmDeltaB670 CL3 and SIVsm543-3E). In the second protocol, mice were immunized with unfixed CP-MAC-infected cells and MAbs were screened for the ability to inhibit cell-cell fusion. In

contrast to MAbs generated against sgp140, the seven MAbs produced using this protocol did not react with Env by Western blotting and were strongly positive by **FACS** analysis, and several reacted preferentially with oligomeric Env. All seven MAbs potently neutralized SIVmac1A11, and several neutralized SIVsmDeltaB670 CL3 and/or SIVsm543-3E. MAbs that inhibited **gp120** binding to CD4, CCR5, or both were identified in both groups. MAbs to the V3 loop and one MAb reactive with the V1/V2 loop interfered with CCR5 binding, indicating that these regions of Env play similar roles for SIV and **human immunodeficiency virus**. Remarkably, several of the MAbs generated against infected cells blocked CCR5 binding in a V3-independent manner, suggesting that they may recognize a region analogous to the conserved coreceptor binding site in **gp120**. Finally, all neutralizing MAbs blocked infection through the alternate coreceptor STRL33 much more efficiently than infection through CCR5, a finding that has important implications for SIV neutralization assays using CCR5-negative human T-cell lines.

L35 ANSWER 5 OF 16 MEDLINE on STN
2000040383. PubMed ID: 10570278. Circulating CD2+ monocytes are dendritic cells. Crawford K; Gabuzda D; Pantazopoulos V; Xu J; Clement C; Reinherz E; Alper C A. (The Center for Blood Research, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, and Departments of Pediatrics, Neurology, and Pathology, Harvard Medical School, Boston, MA 02115, USA.) Journal of immunology (Baltimore, Md. : 1950), (1999 Dec 1) 163 (11) 5920-8. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Low levels of CD2 have been described on subsets of monocytes, macrophages, and dendritic cells. CD2 is expressed on about one-third of circulating monocytes, at levels one-half log lower than on T or NK cells, representing 2-4% of PBMC. **FACS** analysis of CD2+ and CD2- monocytes revealed no significant difference in the expression of adhesion molecules (CD11a/b/c), class II Ags (HLA-DR, -DQ, -DP), myeloid Ags (CD13, CD14, CD33), or costimulatory molecules (CD80, CD86). Freshly isolated CD2+ and CD2- monocytes were morphologically indistinguishable by phase contrast microscopy. However, scanning electron microscopy revealed large prominent ruffles on CD2+ monocytes in contrast to small knob-like projections on CD2- monocytes. After 2 days of culture, the CD2+ monocytes largely lost CD14 expression and developed distinct dendrites, whereas the CD2- monocytes retained surface CD14 and remained round or oval. Freshly isolated CD2+ monocytes were more potent inducers of the allogeneic MLR and more efficiently induced proliferation of naive T cells in the presence of **HIV**-1 **gp120** than did CD2- monocytes. After culture in the presence of GM/CSF and IL-4, CD2+ monocytes were up to 40-fold more potent than monocyte-derived dendritic cells or CD2- monocytes at inducing allogeneic T cell proliferation. These findings suggest that circulating CD2+ and CD2- monocytes are dendritic cells and the precursors of macrophages, respectively. Thus, dendritic cells are far more abundant in the blood than previously thought, and they and precursors of macrophages exist in the circulation as phenotypically, morphologically, and functionally distinct monocyte populations.

L35 ANSWER 6 OF 16 MEDLINE on STN
1999388927. PubMed ID: 10461831. Sendai virus-based production of **HIV** type 1 subtype B and subtype E envelope glycoprotein 120 antigens and their use for highly sensitive detection of subtype-specific serum antibodies. Toriyoshi H; Shioda T; Sato H; Sakaguchi M; Eda Y; Tokiyoshi S; Kato K; Nohtomi K; Kusagawa S; Taniguchi K; Shiino T; Kato A; Foongladda S; Linkanonsakul S; Oka S I; Iwamoto A; Wasi C; Nagai Y; Takebe Y. (AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan.) AIDS research and human retroviruses, (1999 Aug 10) 15 (12) 1109-20. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB We previously described a Sendai virus (SeV)-based expression system for the recombinant **gp120** of **HIV**-1 subtype B (rgp120-B), which has permitted the production of antigenetically and functionally authentic **gp120** at a concentration as high as 6 microg/ml of culture supernatant

(Yu D et al.: Genes Cells 1997;2:457-466). Here the same procedure was successfully applied to the production of **HIV**-1 subtype E **gp120** (rgp120-E). The remarkable production of the proteins by the SeV expression system enabled us to use crude culture supernatants for serological and functional studies of gp120s. The immunological authenticity of rgp120-E was verified by patient sera and anti-V3 loop monoclonal antibodies specific for **HIV**-1 subtypes B and E. CD4-binding properties were corroborated by **FACS** analyses. The rgp120s were then used in an enzyme immunoassay (rgp120-EIA) to detect antibodies in the sera of **HIV**-1-infected individuals, and the performance was assessed in comparison with a conventional V3 loop peptide EIA (V3-EIA). The initial evaluation of a serum panel (n = 164) consisting of 76 subtype E and 88 subtype B sera revealed that the rgp120-EIA was nearly 1000-fold more sensitive than the V3-EIA and was able to detect subtype-specific antibody with 100% sensitivity and with a complete correlation with the genotypes, whereas the V3-EIA failed to detect 9 and 24% of the same subtype E and B sera, respectively. Furthermore, a study employing a panel of 28 international sera with known genotypes (**HIV**-1 subtypes A through F) confirmed the remarkable specificity of this method. An EIA reactivity higher than 1.0 was an unambiguous predictor of **HIV**-1 subtype E and B infections. The data imply the presence of strong subtype-specific epitopes for antibody bindings to these rgp120s.

L35 ANSWER 7 OF 16 MEDLINE on STN
97360027. PubMed ID: 9217055. A human IgG1 (b12) specific for the CD4 binding site of **HIV**-1 neutralizes by inhibiting the virus fusion entry process, but b12 Fab neutralizes by inhibiting a postfusion event. McInerney T L; McLain L; Armstrong S J; Dimmock N J. (Department of Biological Sciences, University of Warwick, Coventry, United Kingdom.) Virology, (1997 Jul 7) 233 (2) 313-26. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The human b12 IgG1, specific for the CD4 binding site of the **gp120** of **HIV**-1, was prepared by recombinant DNA technology. It had a high neutralization rate constant (-3.5 x 10(5) M(-1) sec(-1)), although this is about 10-fold less than the values for the best poliovirus or influenza A virus MAbs. The recombinant b12 Fab neutralized well, with about one-tenth of the activity of b12 IgG. The mechanisms by which b12 IgG1 and its Fab neutralize **HIV**-1 IIIB on C8166 cells have been investigated. Neither inhibited attachment of virus to the target cell as judged by **FACS**, immunofluorescence, and ELISA data. This was controlled using MAb F105, another human IgG1, that did neutralize by inhibiting attachment under our conditions. The interactions of b12 IgG- and Fab-neutralized virions with target cells were compared with those of nonneutralized virus using a number of different techniques (fluorescence dequenching of R18-labeled virions, immunofluorescence of virion gp41 and p24 antigens, and acquisition of resistance to removal of virions from the cell by protease). These and the inhibition of **HIV**-1-mediated cell-cell fusion all demonstrated that b12 IgG neutralized by inhibiting the primary fusion-uncoating mechanism. However, the interactions of b12 Fab-neutralized and nonneutralized virions with C8166 cells were indistinguishable. Thus b12 Fab did not inhibit fusion uncoating, and by inference inhibited a stage of infection that occurs after the entry of the virion core into the cytoplasm. It is therefore possible that b12 IgG kills **HIV**-1 twice over, by fusion-inhibition and by inhibiting the postentry event proposed for the Fab. The mechanism of neutralization of b12 Fab and of other MAbs that neutralize in a similar way and why b12 Fab and IgG neutralize by different mechanisms are discussed.

L35 ANSWER 8 OF 16 MEDLINE on STN
97163431. PubMed ID: 9010251. Escape of **HIV**-1 is associated with lack of V3 domain-specific antibodies in vivo. Schreiber M; Muller H; Wachsmuth C; Laue T; Hufert F T; Van Laer M D; Schmitz H. (Medical Microbiology Section, Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.) Clinical and experimental immunology, (1997 Jan) 107 (1) 15-20. Journal code: 0057202. ISSN: 0009-9104. Pub. country: ENGLAND: United Kingdom. Language: English.

AB This study was performed to analyse correlates of viral escape in AIDS patients. Peripheral blood mononuclear cells (PBMC) from **HIV**- donors were inoculated with AIDS patients' serum to detect neutralization-resistant cell-free virus. Infectious virus was detected by polymerase chain reaction (PCR) and analysed by sequencing the V3 region. The escaped virus species was compared with all V3 virus variants found in the patients' PBMC and plasma. In one patient escaped virus was also compared with variants found in CD4+ T cells isolated by **FACS** from blood, spleen and lymph node. The frequency of the virus variants was determined by cloning and sequence analysis of 20 V3 clones for each PCR amplification. To monitor anti-V3 antibodies by ELISA, each V3 sequence was expressed as fusion with glutathione S-transferase (GST-V3). In our AIDS patients, a V3-directed antibody response against the infectious virus V3 loop was not detectable. In contrast, virus variants unable to infect the donor PBMC in vitro were well recognized by homologous V3-directed antibody. After an interval of 1 year the frequency of these variants clearly decreased, while at the same time the escaped variants grew out and finally represented the predominant viral species both in plasma and PBMC. The infectious variants lacking V3 antibody response were also predominant in CD4+ T cells in spleen and lymph node. Our data indicate that the escape of virus variants is closely related to the lack of V3-directed antibody.

L35 ANSWER 9 OF 16 MEDLINE on STN
97152500. PubMed ID: 9000083. Two neutralizing anti-V3 monoclonal antibodies act by affecting different functions of **human immunodeficiency virus** type 1. Armstrong S J; McInerney T L; McLain L; Wahren B; Hinkula J; Levi M; Dimmock N J. (Department of Biological Sciences, University of Warwick, UK.) Journal of general virology, (1996 Dec) 77 (Pt 12) 2931-41. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Monoclonal antibody (MAb) ICR41.1i (rat IgG2a) is specific for a conformation-dependent epitope of **human immunodeficiency virus** type 1 (**HIV**-1) V3 , and MAb F58 (mouse IgG1) recognizes the peptide IXXGPGR, at the tip of the V3 loop. Both MAbs neutralized **HIV**-1 strain IIIB in C8166 and HeLa-T4(CD4) cells. Neutralization by either MAb did not inhibit attachment of virus to target cells as determined by **FACS** analysis, ELISA or immunofluorescence, and such attachment was absolutely dependent on the availability of CD4 molecules. F58 inhibited virus-induced cell-cell fusion, and reduced internalization of virions in direct proportion to neutralization. In contrast, ICR41.1i had no effect on **HIV**-1-mediated cell fusion or on internalization of virus. It was concluded that MAb F58 neutralized infectivity by inhibiting fusion of the virus with the cell and internalization of the viral core, and that ICR41.1i neutralized by inhibiting a post-fusion-internalization event. The possible mechanism by which a neutralizing antibody binds to the V3 loop and affects the function(s) of structures inside the virion is discussed. Lastly, postattachment neutralization (PAN) was investigated. F58 mediated PAN at 21 degrees C and 35 degrees C. However, ICR41.1i gave PAN at 21 degrees C but not at 35 degrees C, suggesting that a temperature-dependent event affecting the V3 loop had abrogated neutralization. Overall, it appears that antibodies to different epitopes within the V3 loop neutralize by affecting very different functions of the virus.

L35 ANSWER 10 OF 16 MEDLINE on STN
95003953. PubMed ID: 7522715. Specific ligation of the **HIV**-1 viral envelope protein **gp120** on human CD34+ bone marrow-derived progenitors. Arock M; Dedenon A; Le Goff L; Michel A; Missenard G; Debre P; Guillosson J J. (Laboratory of Hematology, Faculty of Pharmacy, Paris, France.) Cellular and molecular biology (Noisy-le-Grand, France), (1994 May) 40 (3) 319-23. Journal code: 9216789. ISSN: 0145-5680. Pub. country: France. Language: English.

AB The precise mechanisms of hematologic abnormalities observed during **HIV** infection remain unknown. In vitro experiments performed by various authors concerning the **HIV** toxicity on bone marrow-derived precursors did not allow them to determine whether this toxicity could be mediated

through direct or non-direct effects, since it is today unclear if **gp120** possesses a direct hematotoxic effect on human bone marrow progenies. The aim of our study was to determine whether labelled **gp120** could specifically bind to the membrane of purified human normal CD34+ cells and to investigate the in vitro effect of the **gp120** on their growth. To answer these questions, human CD34+ cells were purified from normal bone marrow samples, then labelled with monoclonal antibodies directed either against CD4 antigen or CD34 antigen and/or with FITC labelled **gp120** and analyzed by **FACS**. Our results demonstrate the presence of about 5% of CD4+CD34+ cells and of nearly 12% of CD34+**gp120**+ precursors. Together with our results concerning the in vitro inhibitory effect of **gp120** on the growth of the same purified CD34+ precursors, our data demonstrated the direct hematotoxic activity of **HIV**-derived **gp120** and the possible **HIV** infection of hematopoietic progenitors through the interaction of gp 120 with CD34+ cell surface.

L35 ANSWER 11 OF 16 MEDLINE on STN

94063979. PubMed ID: 8244440. **HIV**-1 gp41 binding to human peripheral blood mononuclear cells occurs preferentially to B Lymphocytes and monocytes. Chen Y H; Bock G; Vornhagen R; Steindl F; Katinger H; Dierich M P. (Ludwig Boltzmann Institute for AIDS Research, Innsbruck, Austria.) Immunobiology, (1993 Aug) 188 (4-5) 323-9. Journal code: 8002742. ISSN: 0171-2985. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Based on our findings that **HIV**-1 gp41 independently of CD4 can bind to the human helper T lymphoid cell line H9, B cell line Raji and monocyte cell line U937, we characterized putative binding of **HIV**-1 gp41 to human peripheral blood lymphocytes (PBLs) and monocytes. Using flow cytometry (**FACS**), we demonstrated that the recombinant soluble **HIV**-1 gp41 (sgp41; Env amino acid 539-684) can bind to the normal human peripheral blood mononuclear cells (PBMCs), preferentially to B lymphocytes and monocytes independently of **gp120**-binding sites on CD4 molecules. This binding is dose-dependent. The **HIV**-1 sgp41 binds to blood B lymphocytes and monocytes more strongly than to T lymphocytes. By two-color flow cytometric analysis, we identified that sgp41 can bind 10% of CD4+ T lymphocytes, 11.9% of CD8+ T lymphocytes, 47% of CD19+ B lymphocytes and 44.2% of CD14+ monocytes.

L35 ANSWER 12 OF 16 MEDLINE on STN

93378781. PubMed ID: 8369165. A hidden region in the third variable domain of **HIV**-1 IIIB **gp120** identified by a monoclonal antibody. Laman J D; Schellekens M M; Lewis G K; Moore J P; Matthews T J; Langedijk J P; Meloen R H; Boersma W J; Claassen E. (Department of Immunology and Medical Microbiology, Medical Biological Laboratory TNO, Rijswijk, The Netherlands.) AIDS research and human retroviruses, (1993 Jul) 9 (7) 605-12. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB The third variable domain (V3 domain) of **HIV**-1 **gp120** is involved in virus neutralization by antibody, in determination of cell tropism, and in syncytium-inducing/non-syncytium-inducing capacity. Antibodies are highly specific tools to delineate the role of different V3 amino acid sequences in these processes, and to dissect events occurring during synthesis of **gp120**/160, **gp120**-CD4 interaction, cellular infection, and syncytium formation. We describe here an IgG1 murine monoclonal antibody (MAb), coded IIIB-V3-01, that was raised with a synthetic peptide (FVTIGKIGNMRQAHC) derived from the carboxy-terminal flank of the **HIV**-1 IIIB V3 domain. The binding site of this antibody was mapped to the sequence IGKIGNMRQ, using Pepscan analysis. In ELISA, this antibody binds to E. coli-derived **gp120** from **HIV**-1 IIIB, which is denatured and not glycosylated. The antibody showed no neutralizing activity against **HIV**-1 IIIB, MN, SF2, or RF in a virus neutralization assay and in a syncytium formation inhibition assay. In addition, this antibody did not react with **gp120** expressed on the surface of IIIB-infected MOLT-3 cells in **FACS** analysis. To assess whether the epitope defined by MAb IIIB-V3-01 is hidden on native **gp120**, reactivity of the antibody with SDS-DTT-denatured or DTT-denatured glycosylated **gp120** (CHO cell

produced) was tested. Both these treatments exposed the epitope for binding. From these data we conclude that the epitope defined by MAB IIIB-V3-01 is hidden on glycosylated recombinant **gp120**, and is not accessible on **gp120** expressed on the membrane of **HIV**-1, IIIB-infected cells.(ABSTRACT TRUNCATED AT 250 WORDS)

L35 ANSWER 13 OF 16 MEDLINE on STN
92159651. PubMed ID: 1535985. Antibodies to MHC class II peptides are present in **HIV**-1-positive sera. Zaitseva M B; Moshnikov S A; Kozhich A T; Frolova H A; Makarova O D; Pavlikov S P; Sidorovich I G; Brondz B B. (All-Union Cancer Research Center, Academy of Medical Sciences, Moscow, USSR.) Scandinavian journal of immunology, (1992 Mar) 35 (3) 267-73. Journal code: 0323767. ISSN: 0300-9475. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Seventy-five per cent of sera from **HIV**-1-infected individuals bind to the human B-lymphoma cells bearing the major histocompatibility class II molecule in enzyme-linked immunosorbent assay (ELISA). The binding is caused by the antibodies against the class II molecule present in the serum samples which prevent the interaction of murine anti-HLA.DR monoclonal antibody with B lymphoma in **FACS** analysis. The three highly conserved amino acid sequences in alpha- and beta-chains of the class II molecule and three homologous fragments in **HIV**-1 **gp120** and gp41 were identified by computer search and synthesized. Using these peptides it was demonstrated that 28-48% of **HIV**-positive sera contain antibodies that cross-react with the peptide of **HIV**-1 origin and with the peptide from the class II molecule as well.

L35 ANSWER 14 OF 16 MEDLINE on STN
91142702. PubMed ID: 1996409. Conglutinin binds the **HIV**-1 envelope glycoprotein gp 160 and inhibits its interaction with cell membrane CD4. Andersen O; Sorensen A M; Svehag S E; Fenouillet E. (Department of Medical Microbiology, Odense University, Denmark.) Scandinavian journal of immunology, (1991 Jan) 33 (1) 81-8. Journal code: 0323767. ISSN: 0300-9475. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The highly glycosylated envelope glycoprotein (gp 160) of **human immunodeficiency virus** (**HIV**) interacts with the CD4 molecule present on the membrane of CD4+ cells and is involved in the pathobiology of **HIV** infection. Lectins bind glycoproteins through non-covalent interactions with specific hexose residues. The mammalian C-type lectin bovine conglutinin was examined for its ability to interact with recombinant **gp160** (rgp160) produced in vaccinia virus-infected BHK21 cells. Specific binding of conglutinin to rgp160 was demonstrated by ELISA. The interaction of bovine conglutinin with rgp160 was calcium-dependent, which is characteristic of the binding of a C-type lectin to its ligand, and the binding was inhibited in a dose-dependent manner with N-acetyl-D-glucosamine. Deglycosylation of rgp160 abrogated the conglutinin binding. In addition, conglutinin exerted a dose-dependent inhibition of the binding of rgp160 to the CD4 receptor on CEM 13 cells, as demonstrated by **FACS** analyses. These results indicate that conglutinin may inhibit the infection with **HIV**-1 through its interaction with the viral envelope glycoprotein.

L35 ANSWER 15 OF 16 MEDLINE on STN
90224001. PubMed ID: 1691563. Dextran sulfate and other polyanionic anti-**HIV** compounds specifically interact with the viral **gp120** glycoprotein expressed by T-cells persistently infected with **HIV**-1. Schols D; Pauwels R; Desmyter J; De Clercq E. (Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium.) Virology, (1990 Apr) 175 (2) 556-61. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Eighty to 100% of persistently **HIV**-1-infected HUT-78 cells express the viral glycoprotein **gp120** as demonstrated with anti-gp 120 monoclonal antibody (mAb) and fluorescence-activated cell sorter (**FACS**) analysis. Several polyanionic anti-**HIV** compounds, i.e., dextran sulfate, pentosan polysulfate, heparin, aurintricarboxylic acid (ATA), suramin, and Evans blue, which are known to inhibit the adsorption of **HIV** particles to CD4+

cells, prevented the binding of anti-**gp120** mAb to the persistently **HIV**-1 infected HUT-78 cells. This effect was dose-dependent and reversible. Except for ATA, the polyanionic compounds did not interfere with the binding of Leu3a/OKT4A mAB, indicating that they do not directly bind to the CD4 receptor. Thus, the inhibitory effect of dextran sulfate and its congeners on the interaction of the **HIV gp120** with the cellular CD4 receptor can be ascribed to a specific binding ("shielding") of **gp120**.

L35 ANSWER 16 OF 16 MEDLINE on STN
90218647. PubMed ID: 1691288. Sulfated polysaccharides as potent inhibitors of **HIV**-induced syncytium formation: a new strategy towards AIDS chemotherapy. Baba M; Schols D; Pauwels R; Nakashima H; De Clercq E. (Rega Institute for Medical Research, University of Leuven, Belgium.) Journal of acquired immune deficiency syndromes, (1990) 3 (5) 493-9. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB Multinucleated giant cell (syncytium) formation induced by the interaction between the **gp120** glycoprotein expressed on the surface of cells infected with **human immunodeficiency virus** type (**HIV**-1) and the CD4 receptor of uninfected CD4-positive (CD4+) cells may play an important role in the depletion of T4 lymphocytes in acquired immune deficiency syndrome (AIDS) patients. Using a double fluorescence cell-staining technique and analysis of the cells by the fluorescence-activated cell sorter (**FACS**), we have demonstrated that giant cell formation between persistently **HIV**-1-infected HUT-78 cells and uninfected MOLT-4 cells results in a selective destruction of the uninfected CD4+ MOLT-4 cells. Apparently, bystander CD4+ cells may serve as targets for the killing effect of the **HIV**-1-infected cells, and this killing effect is preceded by fusion between the target (uninfected) and aggressor (infected) cells. Pentosan polysulfate, dextran sulfate, and various other sulfated polysaccharides, but not heparin, have proved to inhibit this cell fusion process and hence protect the target CD4+ cells against destruction by the killer **HIV**-1-infected cells. Azidothymidine does not interfere with this process. Assuming that fusion between **HIV**-infected and uninfected CD4+ cells is a crucial event in the pathogenesis of AIDs, any compounds that specifically interfere with this process may be therapeutically advantageous in the treatment of this disease.

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=> d his

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     FILE 'USPATFULL' ENTERED AT 13:38:06 ON 17 JUN 2004
                E HALLOWITZ R A/IN
L1            7 S E4 OR E5
                E KROWKA JOHN/IN
L2            1 S E3
                E MATLOCK SHAWN/IN
L3            2 S E3 OR E4
L4        33048 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5         3422 S L4 AND (GP120 OR GP160)
L6         2490 S L5 AND (CD4?)
L7          182 S L6 AND (FRET OR RET OR FLUORESCENT RESONANCE ENERGY TRANSFER
L8          182 S L7 AND ANTIBOD?
L9           16 S L8 AND (GP120/CLM OR GP160/CLM)
L10          20 S L7 AND AY<2000
L11          15 S L10 NOT L9
L12        2375 S L6 AND ANTIBOD?
L13        1113 S L12 AND (ANTIBOD?/CLM)
L14         211 S L13 AND (GREEN FLUORESCENT PROTEIN OR GFP OR RENILLA LUCIFERA
L15          19 S L14 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 14:21:56 ON 17 JUN 2004
                E HALLOWITZ R A/AU
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L16            3 S E3
L17            1 S E2

     FILE 'BIOSIS' ENTERED AT 14:24:22 ON 17 JUN 2004
                 E HALLOWITZ R A/AU
L18            8 S E3 OR E4

     FILE 'MEDLINE' ENTERED AT 14:25:54 ON 17 JUN 2004
                 E KROWKA J/AU
L19            9 S E3

     FILE 'USPATFULL' ENTERED AT 14:27:23 ON 17 JUN 2004
L20          800 S L6 AND (PARAMAGNETIC OR MAGNETIC)
L21           39 S L20 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)
L22           19 S L21 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 14:33:58 ON 17 JUN 2004
L23       142285 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L24         6469 S L23 AND (GP120 OR GP160)
L25         2828 S L24 AND (CD4?)
L26            5 S L25 AND (FRET OR RET OR RESONANCE ENERGY TRANSFER)
L27         2811 S L23 AND (INFECTED CELLS OR INFECTIVITY STATUS)
L28            0 S L23 AND (INFECTIVITY STATUS)
L29          445 S L27 AND (GP120 OR GP160)
L30           85 S L29 AND (DETECT? OR DIAGNOS?)
L31            4 S L30 AND (QUANTITATIVE? OR CELL NUMBER)
L32            9 S L30 AND QUANTI?
L33            5 S L32 NOT L31
L34         2391 S L24 AND (MEASUR? OR DETERMIN? OR DETECT?)
L35           16 S L24 AND (FACS OR FLUORESCENT ACTIVATED CELL SORT?)

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 14:51:45 ON 17 JUN 2004
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